Burden of Malaria in Pregnancy in Mali and Impact of Dosing frequency and Antimalarial drug Resistance on the Effectiveness of Intermittent Preventive Therapy in pregnancy in Africa

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By

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Statement of own work

I, Kassoum Kayentao, confirm that the work presented in the thesis is my own, completed under the supervision of Prof. Feiko Ter Kuile. However, some field and laboratory activities were conducted by other colleagues in specific parts of this thesis. The level of contribution of individual is listed in published (chapter 3, 4 & 5) chapters. I have been assisted by Dr. Anna Maria van Eijk for the PAM estimate computation (chapter 2) and by Dr. Steve Taylor and Prof. Abdoulaye Djimde for all the PCR analysis in chapters 6. They have been fully acknowledged in recognition of their contribution.

Signed: _____

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Abstract

For many centuries, malaria has remained the most common parasitic disease in sub-Saharan Africa potentially placing 32 million pregnancies at risk each year. Malaria in pregnancy (MiP) in malaria endemic Africa is mostly asymptomatic or paucisymptomatic, yet associated with maternal anaemia and intra-uterine growth retardation resulting in low birth weight (LBW) which is an important risk factor for infant mortality (chapter 1).

In Mali, several observational studies have determined the risk and consequences of malaria in pregnancy. However, national estimates of the burden of MiP and its potential impact are lacking. This thesis describes the results of a series of surveys conducted in different malaria transmission settings countrywide from 2005 to 2010, to quantify the burden and consequences of MiP in Mali (chapter 2). Results demonstrate that the risk of malaria infection at delivery was generally high ([average prevalence 11.6%]) and showed marked diversity between regions and transmission settings. Coverage of intermittent preventive treatment (IPTp) with sulfadoxine-pyrimethamine (SP) and impregnated treated bednets (ITNs) was low (30.4% and 60.7%) and indicated important miss opportunities for the control of PAM.

To prevent the disease and its consequences in pregnancy, the World Health Organization recommends IPTp using SP and use of an ITN. For IPTp, the recommended regimen consists of at least 2 doses of SP given during the second and third trimesters for HIV negative women and at least 3 doses for HIV-positive women not receiving cotrimoxazole. However, there are concerns that the 2-dose regimen, which provides at most 12 weeks of prophylaxis, leaves many women unprotected for as much as half of the 20-26 pregnancy weeks after quickening. Re-infection with the 2-dose regimen is common, especially during the transmission seasons and amongst women who complete their last dose early in the third trimester. A trial was therefore conducted to compare the standard 2-dose regimen versus 3 doses using SP, hypothesizing that the third dose may add significant benefit over the 2-dose regimen in preventing placental malaria and other birth outcomes (chapter 3). The study concluded that IPTp-SP with 3-doses was superior to the standard 2-dose regimen and resulted in better birth outcomes.

The results of this trial were then combined with 6 similar trials as part of a meta-analysis assessing if 3 or more doses of IPTp-SP are more effective than the current standard 2-dose regimen. The pooled results suggested a marked benefit of adding extra SP doses over the standard 2-dose regimen in both regions of high and low SP resistance as measured by the prevalence of *dihydropteroate synthase* K540E mutations (chapter 4).

Although studies from western Africa favour the use of IPTp-SP, SP resistance is now a serious threat to the longevity of IPTp with SP in parts of eastern and southern Africa where the quintuple *dihydofolate reductase (*N51I, C59L, S108N) */dhps* (A437G, K540E) mutation is saturated in many places. In order to get a better understanding of the impact of SP resistance on IPTp effectiveness, this thesis also determined the *in vivo* response of parasites in asymptomatic parasitaemic pregnant women who received IPTp-SP and the effectiveness of IPTp-SP on birth parameters in West-Africa (chapter 5 & 6) Overall, the study concluded that SP resistance in Mali and Burkina Faso is low and that the IPT-SP is associated with clinically relevant improvements in birth outcomes in Mali.

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Acronyms

ACM	Assumed Control Risk
ACT	Arteminsin Combination Therapy
ANC	Antenal clinic
APR	Adjusted Prevalence Ratio
AZI-CQ	Azithromycin-chloroquine
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CIM	Corresponding Intervention group Median
CONSORT	Consolidated Standards of Reporting Trials
CSA	Chondroitin sulphate-A
DBL	Duffy-Binding-link
DHFR	Dihydrofolate reductase
DHP	Dihydroartemisinin-Piperaquine
DHPS	Dihydropteroate synthase
DHS	Demographic Health Survey
DNA	Deoxyribonucleic acid
FANC	Focused antenatal care
FMPOS	Faculty of Medicine, Pharmacy and Odontostomatology
G1-G2	First or second pregnancy
G3	Three or more pregnancies
GLM	Generalized Linear Model
GLURP	Glutamate Rich Protein
HA	Hyaluronic Acid
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
HRP-2	Histidine Rich Protein-2

IFN_Y	Interferon gamma
IgM	Immunoglobulin M
IPTp	Intermittent Preventive Therapy in pregnancy
IRS	Indoor Residual Spraying
IST	Intermittent Screening and Treatment
ITNs	Insecticide Treated Nets
IUGR	Intra Uterine Growth Retardation
LBW	Low Birth Weight
MiP	Malaria in Pregnancy
MRTC	Malaria Research and Training Center
MQ	Mefloquine
MSP	Merozoid Surface Protein
NMCP	National Malaria Control Program
NNT	Number Needed to Treat
PCR	Polymerase Chain Reaction
pLDH	Plasmodium Lactate Dehydrogenase
PQ	Piperaquine
PTD	Preterm delivery
Primi	Primigravida
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
RBM	Roll Back Malaria
RDT	Rapid Diagnosis Test
RR	Relative Risk
Secundi	Secundigravida
SGA	Small for gestational age
SNPs	Single Nucleotide Polymorphisms
SP	Sulfadoxine-Pyrimethamine

TNF	Tumor Necrosis Factor
UNICEF	United Nations Childrens' Fund
WBCs	White Blood Cells
WHO	World Health Organisation

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Chapter 1: General introduction

1. Chapter 1: General introduction

1.1 Epidemiology and burden of malaria during pregnancy

A long time before the discovery of the malaria parasite in 1880 by Alphonse Laveran (Bockarie et al., 1999), pregnant women have been burdened by malaria. However, the association between the two conditions was described only since the early 20th century (Desai et al., 2007). In the first quarter of the 21st century, malaria continues to place approximately 125 million pregnant women at risk each year who live in malaria endemic areas, of whom 32 million live in sub-Saharan Africa (Dellicour et al., 2010). Five parasites species (*Plasmodium falciparum*, *P. malariae*, *P. vivax*, *P. ovale* , and *P. knowlesi*) were described to infect humans (White, 2008). However, recently infection with *Plasmodium cynomolgi* has been also found in a female patient of 39 year old from east coast of Peninsular Malaysia (Ta et al., 2014). Except *P. vivax* and *P. falciparum* which are the most commonly implicated in the occurrence of adverse pregnancy outcomes (Dellicour et al., 2010), the harmful effect of the other four species in pregnancy are not known. The burden is greatest with the latter which is unfortunately the most prevalent in sub-Saharan Africa (Dellicour et al., 2010). It is estimated to account for 25% of placental infections at the time of delivery, 26% of cases of severe anaemia and a 20% incidence of low birth weight (LBW) in stable transmission areas of Africa (Desai et al., 2007).

1.1.1 Vulnerability of pregnant women

The risk and consequences of the disease in pregnant women relies on a myriad of factors. The vulnerability of pregnant women to disease is due to their immunity impairment compared to non-pregnant adult women. Thus, women become more susceptible to malaria infection during pregnancy with a higher risk of disease and death in both the mother and her foetus.

A study conducted in the Democratic Republic of Congo found more infection among pregnant women than non-pregnant women of child bearing age with 37.2% and 30.4%, respectively (Taylor et al., 2011b).

Compared to their counterpart non-pregnant women, pregnant women are more attractive to mosquitoes (Lindsay et al., 2000, Ansell et al., 2002). In the Gambian

population, Lindsay and colleagues demonstrated that pregnant women were more attractive to *An. gambiae* mosquitoes than their counterpart non-pregnant women under an untreated bednet. The same study showed that the number of mosquitoes entering bednets each night was 1.7-4.5 times higher in the pregnant group ($p = 0.02$), and that pregnant women also received a higher proportion of bites under the bednets than did non-pregnant women (70% vs 52%, $p = 0.001$). The authors suggested that physiological and behavioural changes occurring during pregnancy could be partially responsible. Thus, this study clearly demonstrates that pregnant women are more exposed to malaria parasites than other women.

This vulnerability of malaria in pregnancy is also more important among women in their first and second pregnancies who have not yet developed adequate pregnancy-specific immune responses against the subpopulation of parasites that sequester the placenta (Fried and Duffy, 1998). Thus, they are more prone to the deleterious effects of malaria than age-matched multigravid women after exposure during previous pregnancies and are partially protected against the deleterious effects of these parasite strains that can sequester in the placenta. However this gravidity dependence depends on the level of acquired immunity obtained after repeated infections and thus level of malaria transmission.

1.1.2 Clinical implications in high or stable transmission settings

Though the vast majority of pregnant women infected are asymptomatic (Desai et al., 2007), malaria can result in LBW and maternal anaemia (Guyatt and Snow, 2004), all of which act individually or in concert with each other to increase maternal, neonatal and infant mortality (Katz et al., 2013).

1.1.2.1 Anaemia

Compared to non-pregnant women, anaemia is more common in pregnant women for several reasons including the diluting effects of the increased intravascular volume during the second trimester, as well as the increased demand on iron and folate stores (Ayoya et al., 2006). Among the other important risk factors in sub-Saharan Africa that include HIV infection, inadequate nutrition, heamoglobinopathies and hookworm infestation, the contribution by malaria is substantial. Of the 5-10% of pregnant women

who suffer from maternal severe anaemia, malaria is believed to be the main cause in 26% (Desai et al., 2007, Rogerson et al., 2007b). Maternal severe anaemia increases the mother's risk of death especially at the time of delivery. It is estimated that in holoendemic malarious areas where 5% of pregnant women have haemoglobin levels <7 g/dL, there will be 9 maternal deaths related to severe malaria anaemia per 100 000 live births to primigravida (Brabin et al., 2001).

1.1.2.2 LBW

Low birth weight (LBW) is one of the main determinants of perinatal and infant mortality (Katz et al., 2013, Marchant et al., 2012, Slyker et al., 2014) and has strong association with childhood growth, cognition, and disability (Huang et al., 2007, Guyatt and Snow, 2004). Although LBW is multifactorial (Huang et al., 2007), the contribution of malaria as a cause of preterm delivery and intrauterine growth retardation is remarkable in high malaria endemic areas of Africa (Rogerson et al., 2007b, Guyatt and Snow, 2004). The risk of LBW is doubled in women with placental malaria compared to those without. This is more pronounced in primigravida than multigravida (Desai et al., 2007). In sub-Saharan Africa, 1 in 5 LBW deliveries are attributable to malaria, corresponding to 35% of preventable LBW deliveries in women of all gravida (Taylor et al., 2011b). In the Democratic Republic of Congo, among 880,415 first and second pregnancies, the number of births with LBW was estimated to be 169,704 annually of which 49,214 (29.0%) were preventable if universal coverage with intermittent preventive treatment in pregnancy (IPTp) and insecticide treated nets (ITN) could be achieved (Taylor et al., 2011b).

The impact of malaria induced LBW on newborns and infants' health is particularly alarming. From different reports, it contributes every year to 11.4% of neonatal deaths and 17 infants' deaths per 1,000 live births in Africa (Desai et al., 2007). LBW contributes to at least 13% of all infant deaths each year and the overall perinatal mortality endorsed by malaria was reported to be 25-80/1,000 per year (van Geertruyden et al., 2004).
Other authors reported that infant mortality is three times higher for LBW babies than for those of normal weight, and that neonatal mortality is nine times more with LBW babies than those of normal weight (Guyatt and Snow, 2004). In East Africa, more recent reports suggested more impact of LBW on neonatal mortality (Katz et al., 2013, Marchant et al., 2012). Some reports indicated that LBW is a predictor for poor neurosensory,

cognitive, behavioural development, school performance and academic achievement (Agarwal and Lim, 2003, Corbett and Drewett, 2004).

1.1.3 Clinical implications in low, unstable endemic settings

In these settings, women have little exposure to malaria infections and thus relatively little acquired adult and pregnancy-specific immunity and all ranks of pregnant women are therefore at risk. *P. falciparum* infections are more likely to result in symptoms including fever, headache, vomiting, abdominal and joint pains which, if not treated can progress rapidly to severe malaria leading to death of the mother or foetus (Desai et al., 2007). Although the prevalence of malaria is low in these settings, the overall maternal deaths attributable to malaria are roughly similar to that reported for high transmission areas. Outside Africa, this was reported to be 0.6-12.5% and 0.5-23.0% in low and high transmission settings, respectively. In these settings, infection with *Plasmodium vivax* is also an important risk factor for maternal anaemia and LBW (Desai et al., 2007).

The prevalence of low birth weight was reported to be 9.3% and 16% in low endemic areas of sub-Saharan Africa and outside Africa, respectively (Desai et al., 2007). Compared to stable transmission, the prevalence of premature delivery is more important and was found to be the leading cause of LBW. Though placental malaria infection is not a first line burden in these settings, it was associated with LBW, stillbirth, and preterm delivery in the few existing studies in Asia (Rijken et al., 2012) and sub-Saharan Africa (Desai et al., 2007).

1.1.4 Effect of malaria on infant outcomes

As in the mother, malaria impacts seriously on her offspring at different stages of its growth. The importance of this deleterious effect relies also on the transmission intensity, gravidity of the mother and other factors such as HIV and malnutrition (Desai et al., 2007, Rogerson et al., 2007b). In low endemic areas where pregnant women are less immune against malaria, acute malaria (fever, vomiting, nausea, headache, abdominal pain) is associated with preterm delivery and abortion and stillbirth (Nosten et al., 2004). Maternal malaria in high endemic area is predominantly indirectly associated with infant mortality through a reduction in birth weight through intrauterine growth retardation and with neonatal mortality through prematurity (Steketee et al., 2001, Guyatt and Snow, 2004). In nine studies conducted in sub-Saharan Africa, regardless of

gravidity, placental malaria doubled the risk of stillbirths (van Geertruyden et al., 2004). Clinical trials with IPTp-SP and ITNs have also shown that successful malaria control can substantially curtail foetal loss (abortions and stillbirths) by as much as 33% and perinatal mortality by 27% (Gamble et al., 2007, Garner and Gulmezoglu, 2006). Thus, the contribution of malaria to perinatal death is considerable in sub-Saharan Africa, even though the vast majority of maternal infections in these endemic areas remain asymptomatic or pauci-symptomatic.

Malaria exposure *in utero* may also result in modulation of the immune responses immune sensitisation of the developing fetus (Malhotra et al., 2009). Several studies have shown that the risk of anaemia and malaria in childhood was greater among infants born from mothers with infected placentas in Kenya (Malhotra et al., 2009), Cameroon (Le Hesran et al., 1997), Tanzania (Mutabingwa et al., 2005) and in Gabon (Schwarz et al., 2008).

Compared to non-malarious areas, foetal anaemia is more common in malaria endemic areas suggesting that malaria can be an important contributing factor (Brabin et al., 2004a, Rogawski et al., 2012, Brabin, 1992).

In high endemic areas, a high prevalence of foetal anaemia was observed with an increased risk of high parasite density at delivery and placental malaria based histology (Brabin et al., 2004b). Another contributing factor was birth during the rainy season (Brabin et al., 2004a).

Malaria may contribute to 8-14% of LBW (Steketee et al., 2001), and it has been hypothesized that malaria in pregnancy indirectly may have long term implications through poor neurosensory, cognitive, and behavioural development, as well as potentially poorer school performance and academic achievement (Guyatt and Snow, 2004).

1.2 Pathophysiology of malaria in pregnancy

The mechanism of LBW and malaria is complex, with intrauterine growth retardation (IUGR) and preterm delivery (PTD) as the main pathophysiological pathways. Acute infection and high parasitaemia are associated with PTD whereas chronic placental inflammatory infiltrates are especially associated with IUGR (Foetal growth retardation) (Rogerson et al., 2007b). In foetal growth retardation (FGR) there is an increase in

inflammatory cytokines such as TNF and IL-8 whilst in PTD a reduction of these cytokines may occur (Rogerson et al., 2007b, Melville and Moss, 2013). Different authors reported that chronic parasite infection in the placental blood and the associated cellular immune response may result in consumption of glucose and oxygen that would have gone to the foetus; and that some infected placentas have evidence of thickening of the cytotrophoblastic membranes, which may interfere with nutrient transport (Guyatt and Snow, 2004). Erythrocytes infected with *P. falciparum* congregate in the maternal placental vascular space, where parasites replicate. Malaria infected placentas are frequently observed to carry antibodies, cytokines, and macrophages which are indicative of an active immune response. This immune response may stimulate early labour, though the precise effect of malaria-parasitized placentas on prematurity is not clear (Guyatt and Snow, 2004).

Malaria during pregnancy relies mainly on the infection of placenta by *P. falciparum* which can sequester in the placenta. During *P. falciparum* infection, sequestration of infected erythrocytes (IEs) to endothelial cells in the brain or other organs is mediated by receptors such as CD36 and ICAM-1. However in the placenta, IEs sequestration occurs through adhesion to chondroitin sulphate A (CSA) and, to a lesser extent, to hyaluronic acid (HA), both of which are expressed by syncytiotrophoblast cells that line the placental intervillous spaces. CSA binding IE, unlike other IE, adsorb IgM, which may also promote sequestration (Brabin et al., 2004b, Rogerson et al., 2007b). As women have little or no immunological exposure to these parasites subpopulations prior to their first pregnancy, this makes primigravida women particularly vulnerable to the placental infection and its adverse consequences (Fried and Duffy, 1998). Specific immunity conferring protection against these parasite subpopulations in subsequent pregnancies occurs through the development of anti-adhesion antibodies and / or variant-specific agglutinations antibodies (Fried and Duffy, 1998, Scherf et al., 2001).

Plasmodium falciparum erythrocyte membrane protein and CSA: implication in the immunity of malaria during pregnancy

The immunological basis of this gravidity dependence consists of the unique ability of *P. falciparum* to insert proteins, functioning as adhesins, into the membrane of the infected erythrocytes (IEs) including those infecting the placenta. These proteins called PfEMP1

proteins support *P. falciparum* parasites to sequester in the host microvasculature, thereby avoiding clearance by the spleen system (Tembo and Montgomery, 2010, Fried and Duffy, 1996).

In pregnant women where a distinct subpopulation of *P. falciparum* parasites is sequestered in the placenta, the VAR2CSA protein, which is a member of the PfEMP1 family and present in the intervillous space of the placental syncytiotrophoblast, has been implicated to be responsible for the binding of IEs to the placental tissue (Fried and Duffy, 1998). Acquired protective immunity to placental malaria is developed over successive pregnancies and is correlated with the buildup of anti-VAR2CSA antibodies capable of blocking the VAR2CSA-chondroitin sulfate A interaction. Studies demonstrated that primigravida at the beginning of their first pregnancy do not yet possess these protective antibodies that inhibit CSA-specific IE adhesion, whereas many multigravida who have been exposed to malaria in previous pregnancies have high levels of such antibodies (Fried et al., 1998). VAR2CSA has therefore been the focus of the development of a vaccine for malaria in pregnancy. Available evidence indicates that a vaccine based on VAR2CSA that can inhibit IE adhesion to CSA in the placental intervillous space and opsonize VAR2CSA-positive IEs for phagocytosis would markedly reduce mortality and morbidity from malaria in pregnancy (Hviid, 2011).

1.3 Diagnosis of malaria during pregnancy

As malaria is a devastating disease during pregnancy, its diagnosis is important. Current diagnostic tools available for clinical practice are based on clinical symptoms, microscopy and rapid diagnostic test (RDTs). Other diagnostic methods based on DNA detection are more of research interest.

In malaria endemic countries, malaria symptoms in pregnant women (fever, headache, joint pain, vomiting, abdominal pain, etc) can result from other infectious diseases or even pregnancy itself. This renders the diagnosis difficult and can drive to wrong therapeutic decision as many women may receive unnecessary antimalarial treatment in the absence of infection. In Mali, during the rainy season, in a semi urban area, 60% of sick pregnant women with negative blood smear microscopy received unnecessary

quinine (Kassoum Kayentao, personal communication). A similar observation was made in Mozambique (Bardaji et al., 2008).

Light microscopy of stained thick or thin blood smear is viewed as the gold standard for the diagnosis of malaria in pregnant women for case-management. The sensitivity of this method in expert hands is about 10 infected erythrocytes per µl blood. It also allows for assessment of parasites species, which is best done on the thin film. Although this method is the most widely available because of cost, it requires well trained and experienced technicians.

RDTs are based on the detection of malaria parasite soluble antigens that include *Plasmodium falciparum* histidine-rich protein 2 (HRP2) and plasmodial aldolase and plasmodial lactate dehydrogenase (pLDH). The better quality RDTs have the advantage over microscopy of being able to reliably detect relatively low-density infections in the peripheral blood of pregnant women with similar levels of sensitivity that can be achieved with expert microscopy (Kattenberg et al., 2012) . In addition RDTs may be able of detecting placental malaria in peripheral blood (Uneke, 2008, Kattenberg et al., 2012). However, the widespread use of RTDs is not without its challenges. They tend to be expensive ($0.7 to 1 USD per test), have to be stored at room temperature and their accuracy varies widely between brands and in untrained hands are subject to high rates of false positives, and false negatives results (Faye et al., 2013, Mouatcho and Goldring, 2013, McMorrow et al., 2011).

PCR methods are generally considerably more sensitive than microscopy or RDTs (Mockenhaupt et al., 2006, Mens et al., 2010, Kattenberg et al., 2011). These methods based on parasite DNA detection have the advantage of being able to detect sub microscopic malaria infection (‘absence’ of detectable parasites by microscopy). However, PCR does not detect all cases in which parasites are identified by microscopy or placental histology. This is more frequent with *P. vivax* than with *P. falciparum* (Barker et al., 1992). Also, although this method can process more than a thousand samples per day by a single technician, high volume PCR is an unrealistic alternative for routine malaria diagnosis in pregnant women for point of care.The clinical meaning of sub-microscopic

infections is not yet clear, with an association detected with adverse birth outcome in some but not all studies (Fried et al., 2012).

The gold standard for malaria diagnosis in the placenta is placental histology. It has the advantage over the previous methods of showing signs of active infection (presence of infected erythrocytes in the intervillous space), chronic or past infection (malaria pigment); or both (Uneke, 2008). The main value is for research, not for point of care because of its high cost, requirement for skilled labour, and because the diagnosis is only made after delivery, when it is typically too late to have clinical implications.

1.4 Control of malaria during pregnancy

To tackle the adverse consequences of malaria during pregnancy, the World Health Organization (WHO) recommends a three-pronged approach consisting of intermittent preventive therapy (IPTp) with sulfadoxine–pyrimethamine (SP), insecticide treated nets (ITNs) for prevention, and case management of malaria and anaemia (WHO/AFRO, 2004). The deployment of these interventions relies on many factors including the level of malaria transmission, HIV status, and SP-resistance.

1.4.1 Prevention

1.4.1.1 Intermittent preventive treatment using SP

IPTp is defined as the provision of a curative treatment dose of an effective antimalarial drug at predefined intervals during pregnancy as an integrated part of routine antenatal care. Using SP, IPTp has been introduced as an alternative to weekly CQ chemoprophylaxis which had been the mainstay for malaria prevention during pregnancy since the 1950s (Briand et al., 2007). The safety and efficacy of this strategy has been tested during many randomised controlled and quasi-randomised trials conducted in eastern, southern and western Africa (Kayentao et al., 2013, Ter Kuile et al., 2007). Its introduction started in East Africa in mid 1990s before reaching West Africa in the early 2000s (Van Eijk et al., 2011, van Eijk et al., 2013). IPTp during pregnancy provides intermittent clearance or suppression of existing asymptomatic infections from the placenta (the treatment effect) and because SP is slowly eliminated it may prevent new infections from occurring for several weeks by maintenance of suppressive drug levels (the post-treatment prophylactic effect) (White, 2005). For malaria endemic areas, WHO

recommends that IPTp-SP is given at each scheduled ANC visit, starting as early as possible in the second trimester until delivery, provided that the doses are given at least one month apart (WHO, 2013).

In areas where HIV prevalence is more than 10%, three doses were recommended in all women; no IPTp-SP is required for HIV infected pregnant women on co-trimoxazole prophylaxis for the prevention of opportunistic infections (WHO/AFRO, 2004). The effectiveness of this strategy has been challenged by an alarming rate of SP resistance in eastern and southern Africa (Naidoo and Roper, 2011) and the low coverage of 2 doses (Van Eijk et al., 2011). As previously reported (van Eijk et al., 2008, Peters et al., 2007, Ouma et al., 2006), SP is considered safe after quickening. SP can be given together with folate at a daily dosage of <1 mg (Mbaye et al., 2006a).

1.4.1.2 Insecticide treated nets

In addition to the IPTp strategy, ITN use is also recommended as part of the ANC package to be provided during pregnancy. Its benefit on birth outcomes had been proven in clinical trials (Gamble et al., 2007). In addition, its use has been associated with a reduction in childhood morbidity from malaria (ter Kuile et al., 2003). Thus, this tool used throughout pregnancy and the postpartum period has been promoted to combat pregnancy associated malaria. Similar to IPTp-SP, ITN coverage was also found to be modest despite the wide deployment of international efforts. A review analysis reported coverage between 0.4% (Guinea) and 60.3% (Rwanda) in pregnant women (Van Eijk et al., 2011). This coverage for many sub-Saharan African countries has improved with the recent support from international donors (van Eijk et al., 2013).

1.4.1.3 Indoor residual spraying (IRS)

In addition to ITNs, indoor residual spraying (IRS) has become an increasingly important component of vector control of malaria. Both strategies alone or in combination have shown to have significant impact on malaria indices (Zhou et al., 2013). IRS is now supported by the Global Fund to fight HIV/AIDS, Tuberculosis and Malaria, along with other donors and many countries are embarking on this strategy. However, there is a concern that IRS can potentially affect the coverage of ITNs in pregnant women because of resource competition (Van Eijk et al., 2011). Another concern of this strategy is malaria

vector resistance to common insecticides, especially in areas that use similar insecticide classes for both IRS and ITNs (Tangena et al., 2013, Protopopoff et al., 2013)

1.4.2 Case management

1.4.2.1 Malaria

For symptomatic malaria in pregnancy, the emphasis is on prompt and effective case management of the malaria infection and any concomitant anaemia. This is especially important in low transmission areas where women have little acquired immunity, and where acute uncomplicated malaria in a pregnant woman can rapidly change to severe disease and to death. Because of safety concerns, antimalarial treatments used rely on gestational age, the stage of the disease and the *Plasmodium* species (WHO, 2010). An exception is the treatment of severe malaria in pregnancy when the primary objective is to save the mother's life.

First trimester

Quinine, chloroquine, clindamycin and proguanil can be used without safety concern at recommended doses. For *P. falciparum* uncomplicated malaria, quinine plus clindamycin for 7 days (or quinine monotherapy if clindamycin is not available) is the recommended treatment. If this treatment fails, clindamycin plus artesunate for 7 days is indicated. For complicated malaria, a parenteral antimalarial should be given to pregnant women in full doses without delay. In the first trimester, the risk of hypoglycaemia is lower and the uncertainties over the safety of the artemisinin derivates are greater. However, weighing these risks against the evidence that artesunate reduces the risk of death from severe malaria, both artesunate and quinine can be considered as an option until more evidence becomes available (WHO, 2010).

Second and third trimester

For the treatment of severe malaria in the 2nd and 3rd trimester, parenteral artesunate is preferred over quinine, because quinine is associated with recurrent hypoglycaemia. Artemisinin-combination therapy (ACT) can be recommended for uncomplicated malaria, and if these are not available, an artesunate plus clindamycin given for 7 days or quinine plus clindamycin given for 7 days can be considered as alternatives. All ACTs are recommended in the 2nd and 3rd trimester, although by 2009,

there was relatively little experience with dihydroartemisinin + piperaquine (DHA+PPQ) as first line treatment (WHO, 2010), with the exception of parts of Indonesia where DHA+PPQ has been used as drug of choice in the 2nd and 3rd trimester since 2005 (Poespoprodjo et al., 2011)

For *P. vivax* treatment, chloroquine is the drug of choice in areas where the parasites are sensitive to the drug. ACT is recommended in areas where *P. vivax* is resistant to chloroquine (Nosten et al., 2007).

1.4.2.2 Anaemia

The management of anaemia is part of the routine antenatal clinic package administered to pregnant women at booking. This includes iron and folate supplementation. As iron deficiency is common in pregnant women, weekly iron supplementation is required throughout the pregnancy period. The standard antenatal dose to prevent recurrence of anaemia is 30-60 mg of elementary iron –plus 0.4 mg of folic acid daily (WHO, 2013). If a woman is diagnosed with anaemia, WHO recommends treatment with 120 mg elemental iron daily, given in two separate doses of 60 mg and 0.4 mg folic acid supplementation until her haemoglobin concentration rises to normal. Folic acid at a dose of 5 mg is not recommended with SP-IPTp (WHO, 2013).

1.5 Challenges to Intermittent preventive treatment using Sulfadoxine-pyrimethamine to prevent malaria in pregnancy

IPTp-SP is the mainstay for malaria prevention in pregnant women in sub- Saharan Africa. However, although a very convenient and widely deployed strategy, recently it has faced multiple challenges including an alarming increase in SP resistance, especially in eastern and southern Africa (Harrington et al., 2011). Other challenges include the modest coverage of IPTp 2 and 3 doses of SP (van Eijk et al., 2013). In addition, there is no suitable replacement to SP for IPTp in terms of its costs, tolerance, ease of administration and safety profile.

1.5.1 Resistance to SP and alternative drugs to SP

1.5.1.1 Resistance to SP

SP is an antifolate drug that blocks two key enzymes in the folic acid biosynthetic pathway. Folic acid is needed for the biosynthesis of purines and pyrimidine and hence

DNA synthesis and cell multiplication. Pyrimethamine is a synthetic diaminopyridine that binds to and inhibits the bifunctional enzyme dihydrofolate reductase-thymidylate synthase of plasmodia. This enzyme interrupts nuclear division which results in subsequent death of the cell (Oyibo and Agomo, 2011). Sulfadoxine has a structure analogue of p-aminobenzoic acid (PABA). It is a long-acting sulphonamide that competitively inhibits dihydropteroate synthase (DHPS), an enzyme that is necessary for the conversion of PABA to folic acid. This enzyme is also a component of the folate metabolic pathway and is upstream of DHFR (the enzyme that is targeted by pyrimethamine).

Although the fixed combination of pyrimethamine and sulfadoxine offers a two-step synergistic blockage of plasmodial division, the mechanism which it exerts in IPTp is not clearly understood. However, there is a suggestion of a treatment effect by providing intermittent clearance or suppression of existing asymptomatic infections, and a prophylactic effect by preventing new infections from occurring for several weeks because of the maintenance of its suppressive drug levels (Ter Kuile et al., 2007, White, 2005). The long half-life elimination of the drug contributes to this prophylactic effect. The mean elimination half-life of sulfadoxine and pyrimethamine is 169 h (range: 100-213 h) and 111 h (range: 54-148 h), respectively (Oyibo and Agomo, 2011). This has been suggested to vary during pregnancy, but evidence is not in favour of dose adjustment of the combined drug (Nyunt, 2009). Such a long half-life elimination of SP is also contributing to the risk that parasites resulting from re-infections are exposed to sub-therapeutic drug concentrations, potentially leading to selection of mutant resistant forms (Watkins and Mosobo, 1993). Resistance to sulfadoxine-pyrimethamine occurs from mutations at specific codons in the dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*) genes of the parasite. With increasing drug resistance, the minimum inhibitory concentration at which parasite growth is inhibited, increases and the time window for drug concentrations to fall below these levels shortens. This results in a progressive shortening of the duration of the suppressive prophylactic effect post-treatment. Parasites with triple *dhfr* mutations have an approximate 1000-fold reduction in susceptibility to pyrimethamine, which translates into a reduction in the duration of post-treatment prophylaxis of 1 month, compromising the efficacy of the 2-

dose regimen, which can have a 3-month interval between doses (Ter Kuile et al., 2007, White, 2005). This has been the case in southern and eastern Africa where SP resistance is increasing to alarming levels (Naidoo and Roper, 2013) to mar the effectiveness of the IPTp strategy (Harrington et al., 2011, Harrington et al., 2009, Naidoo and Roper, 2011). However, in the same areas, other reports suggest that IPTp-SP remains efficacious, even in areas with high SP resistance (Kayentao et al., 2013, Ter Kuile et al., 2007).In western Africa, SP resistance is not yet a major public health problem and trials (Diakite et al., 2011, Valea et al., 2010) and observational studies (Gies et al., 2009, Sirima et al., 2006, Vanga-Bosson et al., 2011) demonstrated good sustenance in improving birth outcomes. Good efficacy results in pregnant women and infant were also reported by other authors (Coulibaly et al., 2006, Dicko et al., 2010, Tekete et al., 2009).

This marked variability of SP resistance and the varying impact on birth parameters in African regions, appeal for critical monitoring of SP resistance and its impact on IPTp effectiveness. Such a monitoring tool is being developed by the Malaria in Pregnancy Consortium in collaboration with the World Health Organization.

1.5.1.2 Alternative drugs to SP for IPTp

In the meantime, priority research is underway to look for alternative drugs to SP for IPTp or alternative strategies to IPTp for the control of malaria in pregnancy. Proposed alternatives are discussed below.

Mefloquine (MQ):

Mefloquine (MQ) is a potential candidate to replace SP for IPTp because of its long half-life, possible single dose administration, and efficacy profile against *P. falciparum*, although conflicting results on its safety exist related to risk of stillbirths associated with melfoquine in some of the earlier studies in pregnancy (Briand et al., 2007, Nosten et al., 2007). More recently in Benin, IPTp with MQ showed similar pregnancy outcomes as IPTp-SP, while being more effective in reducing placental and clinical malaria, and maternal anaemia. However, its tolerability profile has led to reservations for this drug for IPTp (Briand et al., 2009, Chico and Chandramohan, 2011b), potentially compromising its large scale use. Members of the Malaria in Pregnancy Consortium are conducting a large scale study to further explore the efficacy, safety, and tolerability of single and split

dose MQ for IPTp (ClinicalTrials.gov identifier NCT00811421). Its combination with artesunate (AS) is also being evaluated in several case-management (treatment) studies in Africa and Asia (Takem and D'Alessandro, 2013)

Dihydro-Artemisinin (DHA)-Piperaquine (PPQ):

This combination is used for malaria treatment in pregnant women in the western Pacific and is now under investigation as treatment option and as IPTp in studies in Africa and Asia (NCT00852423, NCT01054248, and MCTO131113). The combination may have an important role to play as IPTp thanks to its good tolerability and the long terminal elimination half-life of piperaquine (PPQ) (14 days (range of 10 to 18) in children, and 23 days (range of 19 to 28 days) in adults (Dorsey et al., 2007, Zongo et al., 2007) may ensure 4 to 6 weeks post-treatment prophylaxis, while the active rapid antimalarial compound of dihydro-artemisinin (DHA) helps to ensure radical cure of any existing infections among IPTp recipients. Pharmacokinetic studies with DHA-PPQ in pregnancy have also recently been completed and suggests that the same dose can be used in pregnant women as in adults (Tarning et al., 2012) Though preliminary findings on the cure rates and pharmacokinetics are reassuring, the published data on its safety and tolerability in pregnant women remains limited (Davis et al., 2010), although significant operational experience exists in Indonesia where DHA-PPQ has been the national first line therapy in the 2nd and 3rd trimester since 2010, and in Papua-Indonesia since 2005. The combination is under investigation as IPTp in trials in Indonesia and Kenya conducted by members of the Malaria in Pregnancy Consortium (ClinicalTRials.gov identifier NCT01669941 and NCT01231113).

Safety of DHA-PPQ: A trial with monthly and 2-monthly IPT with DHA-PPQ conducted in non-pregnant Thai adults showed that 9 full 3-day treatment courses with DHA-PPQ has a good tolerability, safety, and effectivity profile (Lwin et al., 2012). The study also suggested that for effective prevention of malaria, DHA-PPQ should be given monthly in order to achieve steady state concentrations above the minimum inhibitory concentrations and sustained prophylactic levels (Lwin et al., 2012).

PPQ is well tolerated. Side effects in adults include transient drops in haemoglobin by day 7 (seen with all artemisinins), headache, weakness and fever. The main safety concerns

with PPQ relate to its dose-dependent QTc prolongation. QTc prolongation has been confirmed in clinical trials, but these were mild and similar to many other anti-malarials (Mytton et al., 2007), and there is no indication from clinical data signalling that it is associated with clinically significant arrhythmias (Keating, 2012). This is consistent with recent in vitro models which confirmed that despite mild QTc prolongation the potential cardiac proarrhythmic risk with PPQ is low and similar to that observed with lumefantrine (the long-acting component in Coartem), and lower than for chloroquine. This study concluded that DHA-PPQ does not appear to induce potential torsadogenic effects in vitro which could result in life threatening abnormality of heart rhythm (Keating, 2012).

To minimise QTc prolongation the manufacturer of DHA-PPQ advises patients to take the first day's dose approximately three hours after meals as fatty food can increase the absorption of PPQ. However, the trial providing DHA-PPQ every 2 months or monthly did not find any relationship between drug levels and whether DHA-PPQ was provided with a small amount of fat (200-ml carton of chocolate milk containing 6.4g of fat to be taken with each dose) or without (Lwin et al., 2012), There was no difference at any time point in PPQ concentrations between the group that received DHA-PPQ with fat and the group that received it without fat (Lwin et al., 2012), consistent with observations by others that this amount of fat does not increase exposure to PPQ (Annerberg et al., 2011). More recently a detailed study in healthy volunteers showed that much higher concentration of fat provided by a full high-fat meal (obtained from McDonalds) containing 54g of fat approximately doubles the oral bioavailability of PPQ relative to the fasting state in health volunteers. Importantly, this method of PPQ administration did not produce significant metabolic or cardiovascular effects, the side effects, postural blood pressure changes, electrocardiographic corrected QT interval, serum glucose, and other biochemical and haematological indices were similar in the fasting and fed states over 28 days of follow-up (Sim et al., 2005).

Thus, overall it appears that DHA-PPQ is well tolerated and can be given with small amount of food, although drug levels were also effective when given to fasting patients (Mytton et al., 2007).

Safety of DHA-PPQ in pregnancy

Studies in Indonesia found DHP to be very effective in the treatment of malaria in pregnancy (Poespoprodjo, 2010). In 2006, it was made first-line policy for the treatment of 2nd and 3rd trimester malaria in southern Papua, and in 2010 this became national policy for malaria endemic areas. Between Thailand and Papua, Indonesia, over 1200 pregnancies exposed to DHP have now been carefully documented and DHP was found to be well tolerated and effective. Formal reprotoxicology studies have not raised concern (Tarning et al., 2012)

Azithromycin-chloroquine

Azithromycin (AZI) is a macrolide antibiotic structurally related to erythromycin that also has antimalarial activities. With a good safety profile, this drug has been used extensively in pregnant women for the treatment of sexually transmitted infection (STIs) (Gray et al., 2001) and also for malaria in pregnancy in combination with SP (as IPTp) or artesunate (as treatment) (Kalilani et al., 2007, Luntamo et al., 2010). Its combination as fixed-dose with chloroquine (CQ) in pregnant women has the advantage of providing opportunities to reduce both malaria and bacterial infections in pregnant women in any trimester (Chico and Chandramohan, 2011a). However, if dose adjustment is not needed for AZI, CQ dosing needs to be given for three days. AZI-CQ was under investigation for IPTp in a multicentre, multi-country, phase III, open-label, randomized trial initiated in 2010 by Pfizer Inc. and the Medicines for Malaria Venture (MMV) in Benin, Kenya, Malawi, Tanzania and Uganda (Chandra S Richa, 2013). However, the trial has been stopped prematurely.

1.5.2 Alternative to IPTp strategy:
Intermittent Screening and Treatment strategy

Explorations are underway to evaluate a new test-and-treat strategy that consists of screening using an RDT and treatment of malaria infection using an effective ACT at scheduled antenatal clinic visits (Intermittent Screening and Treatment, IST). This strategy has been tested in Ghana (Tagbor et al., 2010) and is now under investigation under the auspices of the Malaria in Pregnancy Consortium in four West African countries including Mali, Burkina-Faso, Ghana, and the Gambia (ClinicalTrials.gov

Identifier: NCT01084213). Based on data reported in the Kumasi study, this strategy can be a promising alternative to IPTp with SP, especially for areas with high SP resistance such as eastern and southern Africa (Kayentao et al., 2013, Naidoo and Roper, 2011, Ter Kuile et al., 2007), and potentially in areas with very low malaria endemicity such as Zanzibar (Shakely D, 2013), the Gambia (Ceesay et al., 2012), and northern Mali (Doumbo O., 1991). The translation of such a strategy into programmatic conditions in antenatal clinics can be complex and its implementation will have significant logistical challenges. The success of this strategy as a viable alternative to IPTp-SP relies also on the use of RDTs and their sensitivity to detect low-level, chronic placental infections, and their costs. Thus, whether this strategy is operationally feasible and will be a cost effective alternative to IPT-SP where SP resistance is high, or in areas where malaria is declining remains to be determined.

1.5.3 Low coverage of IPTp-SP

To promote and protect both the mother and her offspring health, IPT-SP has been the mainstay for malaria prevention in pregnant women after the decline of CQ chemoprophylaxis. Because of its effectiveness in improving birth parameters (Kayentao et al., 2013, Ter Kuile et al., 2007), a global effort is needed to increase its uptake.

Although IPTp-SP is the most convenient and most widely deployed strategy, its coverage was found to be modest in the majority of the sub-Saharan African countries (van Eijk et al., 2013). In 2010 Roll Back Malaria (RBM) aimed to insure universal coverage for IPTp-SP (RBM, 2008). However, a comprehensive review suggested that by 2010 only one country (Zambia) had reached the Abuja target of 2-dose coverage of 60% in 2005, despite a very high proportion of women making use of ANCs (median of 90%), which constitute an important distribution point of this intervention. Surprisingly, this coverage was lower in high intensity malaria transmission where it is most needed (Van Eijk et al., 2011).

In Mali, the last demographic health survey (DHS) carried out in 2012-2013 reported a coverage of 2-dose IPTp of 19.9% (DHS, 2012-2013); it was 4% in 2006 (DHS, 2006). This improvement is mainly the result of international programme efforts seen in Mali and this could be similar in many sub-Saharan African countries. Despite these

efforts, the most recent review of IPTp uptake suggested stagnation or even decrease in coverage of IPTp in some countries since the last survey (van Eijk et al., 2013).

Thus, missed opportunities of IPTp-SP uptake were suggested; and important factors include: late ANC attendance, SP stockouts, unclear message on IPTp (timing, drug interactions with SP, empty stomach, etc…), lack of training, and nurse underachievement (Van Eijk et al., 2011, Webster et al., 2013a). Problems with IPTp have been reported at all levels including among health workers, IPT-SP recipients, the community environment, and in the government (Hill et al., 2013).

1.6 HIV and malaria during pregnancy

In sub-Saharan Africa, malaria and HIV are the most prevalent infections (WHO, 2005) which can act individually or in concert with each other to become a significant and complex public health interest (Guyatt et al., 2004). It is estimated that 13.5 million of the world's HIV infected women live in sub-Saharan Africa (Desai et al., 2007) where also 32 million pregnant women are exposed to the risk of malaria (Dellicour et al., 2010). Immunological suppression caused by HIV infection reduces the immune response to parasitaemia and therefore leads to an increased prevalence of asymptomatic and symptomatic attacks of malaria in pregnant women. The overall malaria prevalence in pregnancy that could be attributed to HIV is estimated to be 5.5% and 18.8% in areas with HIV prevalence of 10% and 40%, respectively (ter Kuile et al., 2004). The two diseases act independently to lead to moderate to severe anaemia in pregnancy, stillbirth, preterm delivery, low birth weight and foetal growth restriction (ter Kuile et al., 2004, Muhangi et al., 2007). However, they can also act synergistically as HIV increases the degree to which malaria is associated with maternal severe anaemia and LBW by increasing malaria parasite densities (Desai et al., 2007). As described, the burden of malaria in pregnancy is more prevalent in women in their first or second pregnancy than those with multiple pregnancies in malaria stable transmission areas. However, because of compromised malaria immunity caused by HIV infection, this gravidity specific pattern is suppressed, and the trend of the malaria burden among HIV infected women is similar among all gravidae (Desai et al., 2007).

Plasmodium falciparum infection stimulates HIV-1 replication through the production of cytokines (IL-6 and TNF-alpha) by activated lymphocytes (Oyibo, 2009). It has been shown to increase the potential reservoir for HIV in the placenta by increasing the number of chemokine receptor 5 (+) macrophages (Tkachuk et al., 2001) . Placental HIV-1 viral load is increased in women with placental malaria, especially those with high parasite densities (Rogerson et al., 2003, Oyibo, 2009).

The control of malaria in pregnancy changes with HIV infection. IPTp-SP is not recommended if women are receiving daily co-trimoxazole, whereas 3-dose IPTp is recommended for all pregnant women not receiving co-trimoxazole (WHO/AFRO, 2004).

1.7 Description of study sites

Mali is a large landlocked country in West Africa divided in 8 administrative regions with a wide range of malaria transmission patterns as described earlier (Doumbo O., 1991). The infant mortality is estimated to be 99 per 1000 live births and the under-five mortality rate is 176 per 1000 live births (UNICEF, 2011). Malaria accounts for 38.4% of clinic visits with 1,633,423 cases and 2,331 fatalities reported in 2009.

Malaria transmission is highly seasonal, spans usually from June to December with the peak in September-October and varies importantly by regions. Transmission patterns in Mali were classified *'a priori'* into 5 strata based on the prevalence of prevalence of parasitemia in children aged 2-9 years old (Doumbo O., 1991): 1) in the South and South-West of the country, malaria transmission is holo-endemic with a peak infection prevalence of 70 to 80%and with an annual rainfall total of >1,000 mm and a relatively long rainy season lasting ~6 months and an equally long transmission season; 2) the savannah areas extending between the North and South have an annual rainfall total of ~500 to 1000 mm and a season spanning 3-4 months (Maiga et al., 2010). Malaria transmission in these areas is meso-to hyperendemic with peak infection prevalence of 50-70%; 3) the arid Sahelian areas such as in the Timbuktu region in northern Mali have little rain (<300 mm per year) and very short seasons (2-3 months). Malaria transmission is hypo-endemic or epidemic with a peak infection prevalence not exceeding ~ 5%. Nevertheless, even though the annual rainfall is very low in these arid areas, focal transmission can be maintained throughout the year near permanent water reservoirs

such as ponds and oases (Koita et al., 2012); 4) the bimodal transmission areas: In addition to the characteristics for savannah regions, these areas have virtually year-round transmission due to the combination of seasonal rainfall (from June to October) and irrigation projects for the rest of the year (Sogoba et al., 2007, Ceesay et al., 2012); 5) lastly, in the urban transmission areas such as Bamako malaria transmission is low with peak infection prevalence of ~5-10%.

Our studies were conducted in 9 study sites which have been stratified *a priori* (Doumbo et al, 1991) into high (Bougouni, Kita, and Fana), moderate (San, Djenne, Koro, Sangha), and low (Bamako, Timbuktu) transmission settings (Figure 2. 1 & Table 2. 1 in chapter 2). In addition, the site of Bla in moderate transmission area has served for the conduct of the study discussed in chapter 3.

1.7.1 High malaria transmission areas:

Bougouni: Located (Latitude 11.41, Longitude -7.48) in the South of Mali, 180 kilometres south of Bamako. In Bougouni malaria transmission occurs mainly during the rainy season (May-June to October) with the peak in October. The study described in chapter 2 was conducted from September 2006 to March 2007 in the 3 community health centers and the reference health center of the town of Bougouni. This site was identified for the pilot implementation of IPTp at community level by "Save the Children" described in chapter 6. The population of the urban community of Bougouni was estimated to be 59,679 in 2009 with 2,984 pregnant women. The number of assisted deliveries recorded in the four health structures was estimated to be 1,021 in 2007.

Kita: The study described in chapter 2 and 6 was conducted from July 2009 to January 2010 in the reference health center and the two community health centers of the town of Kita (Latitude 13.05, Longitude -9.48). Located 182 kilometers west of Bamako, this site started the IPTp-SP strategy in 2004 with the support of UNICEF. The population of the commune of Kita is estimated to be 48,947 in 2009 with 2,448 pregnant women. In 2009, the number of assisted deliveries was estimated to be 1,358 in the three health centers where the study took place.

Fana: Located (Latitude 12.77, Longitude -6.95) 130 kilometres east of Bamako, Fana is a semi urban area where malaria transmission is intense, with similar rainfall as

the above sites. The chapter 2 study was conducted from November 2005 to February 2006 in the reference health centre which had received more than 1,500 ANC visits and 869 deliveries in 2006. The frequency of the *Pfcrt* K76T mutants in *Plasmodium falciparum* infections in peripheral blood was 68.8% (11/16) and 100% (16/16) in the placenta (*p = 0.004*). The frequency in peripheral blood of the DHFR N51I mutation was 12.5% (2/16) and 18.8% (3/16) in the placenta (*p=0.12*). The frequencies of the DHPS A437G mutants were similar in both sites 25% (4/16). No DHPS K540E and DHFR 164L mutations were found (Doumbo, 2013).

1.7.2 Moderate malaria transmission areas:

Here the surveys described in chapter 2 and 6 were conducted from September 2006 to March 2007 in the health districts of Koro, Djenne, and San.

Since 2003, the sites of *Koro* (Latitude 13.94, Longitude -3.02) and *Djenne* (Latitude 13.9, longitude -4.55) were part of a UNICEF funded pilot implementation program of IPTp as part of their project on "Child and Mother hood Survivor Project". Both Koro and Djenne belong to the Mopti region. The town of Djenne becomes an island during the rainy season resulting from the annual flood produced by Bani river. In 2009, the population and number of pregnant women was estimated to be ~62,681 inhabitants and 3,135 pregnant women in Koro and 32,944 and 1,650 in Djenne, respectively. In both sites, malaria transmission lasts from June to October with the peak in September.

The site of *San* (Latitude 13.3, Longitude -4.9) also benefitted from the support from UNICEF's IPTp pilot program since late 2004. In the town of San, two surveys had been conducted, the first from September 2006 to March 2007 and the second from July 2009 to January 2010. This area has similar characteristics of rainfall and malaria transmission pattern as Koro and Djenne. In 2009, the commune of San had 68,078 inhabitants and 3,404 pregnant women. The number of ANC visits, and assisted deliveries in the town of San was 2,019 and 1,042 in 2009. This site belongs to Segou region as well as the following site.

The site of *Bla* is located (Latitude 12.95, Longitude -5.75) 320 kilometres east of Bamako in the Segou region. The district has had the support from UNICEF since 2001,

but IPTp implementation only started in 2004. The trial comparing 3 versus 2 doses of SP described in chapter 3 was conducted from April 2006 to March 2008 in the two main health facilities in Bla (Diakite et al., 2011), which serve a population of approximately 39,000 inhabitants.

Sangha (Latitude 14.66, Longitude-3.31) is a rural commune, 800 kilometers north-east of Bamako in the district of Bandiagara, in Mopti region. The commune is known as a centre for traditional religion with many temples and shrines, and as a base for visitors to the local Dogon villages. The commune of Sangha had a population of 32,513 inhabitants in 2009 with an estimated 1,626 pregnant women. Malaria transmission occurs during the rainy season which lasts from July to October with annual rainfall of ~700 mm^3. The prevalence of malaria disease in the general population was 14% in 2008 and entomologic inoculation rate (EIR) measured in Bandiagara was 1.1 infected bites per person year (Yaro A.S., 2003). IPTp-SP implementation started in 2004 with the support of UNICEF. The study described in chapter 2 was conducted from June 2006 to February 2007 in the community health center.

1.7.3 Low or epidemic malaria transmission areas:

In Bamako (Latitude 12.65, Longitude -8.00), two surveys were conducted, the first in January 2005 in the community health centre of Banconi having 11,084 antenatal visits and 3,506 deliveries in 2011, and the second from March to November 2009 in the community health centre of Sabalibougou which recorded 3,498 ANC visits and 1,109 deliveries in 2009. The community health centre covered a population of 72,995 in 2008. Although Bamako is characterized by heavy rainfall (more than 1,000 mm per year), malaria transmission is low because of its urbanisation (Doumbo O., 1991, Pond, 2013). Malaria transmission spans from June to December with a peak in October corresponding to the end of the rainy season.

In the *Timbuktu* region, data were collected from January to March 2005 in the reference health centre of Timbuktu (Latitude 16.77, Longitude -3.00) and Niafunke (Latitude 15.93, Longitude -3.99) which is located 200 kilometres west of Timbuktu by the left side of the river Niger. Timbuktu is part of the Sahara, with an average temperature of 38°C (varying from 25°C to 45°C) in the hot dry season (March, April, and

May) and annual rainfall of ~300 mm (Koita et al., 2012). Malaria transmission is thought to be very low or epidemic (Doumbo O., 1991, Koita et al., 2012). Infections with *P.vivax* have been reported in this area (Bernabeu et al., 2012). Although Timbuktu is indicated to be a semi-arid region (Doumbo O., 1991) as are the other regions in northern Mali, the recent arrival of large irrigation projects permitting rice culture during the dry season has created a massive body of water formed by rice paddies. This can maintain mosquito population abundance after the rainy season to levels needed to sustain malaria transmission during the dry season.

1.8 Thesis objectives

1.8.1 Overall objective

The general objective of the thesis was to quantify the burden of malaria in pregnancy and uptake of IPTp and ITNs in pregnancy in Mali, to assess the efficacy and safety of two versus three or more doses of sufadoxine-pyrimethamine in Africa, and to monitor sulfadoxine-pyrimethamine resistance and its potential impact on IPTp-SP effectiveness on birth parameters in Mali.

1.8.2 Specific objectives

This thesis specifically sets out:

1. To quantify the burden and consequences of malaria in pregnant women in different sites and regions belonging to different malaria transmission entities in Mali (chapter 2)
2. To determine the coverage and use of malaria prevention strategies for malaria in pregnancy in different sites and regions in Mali (chapter 2)
3. To compare the 2-dose IPTp regimen versus 3 doses using SP in preventing placental malaria and other birth outcomes in Mali (chapter 3)
4. To determine whether 3 or monthly doses IPTp with SP is more effective for the prevention of malaria in pregnancy than the 2-dose regimen in Africa and to assess if this is modified by the gravidity, HIV status, or the degree of SP-resistance (chapter 4)
5. To determine the efficacy of SP in providing radical cure and preventing new infection when provided to asymptomatic parasitaemic pregnant women as part of

their first course of IPTp in Mali and Burkina Faso, using a 42-day *in vivo* follow-up (chapter 5)

6. To describe the level of SP resistance as assessed by molecular markers (chapter 5)
7. To assess IPTp-SP effectiveness on birth parameters in 5 health districts of Mali and determine how this is correlated with molecular levels of SP resistance in those same areas (chapter 6)

1.9 Thesis outlines and chapters

This thesis consists of seven chapters including 3 published papers (chapters 3, 4, and chapter 5). The publications are attached in the annex of this thesis.

1.9.1 Chapter 1: General introduction on malaria in pregnancy.

This chapter, as described above provides a background on the epidemiology and burden of malaria in pregnancy, a description of study sites where data were collected, and an overview of the overall aim and the specific objectives of the thesis.

1.9.2 Chapter 2: Mapping the risk of malaria during pregnancy in different transmission settings in Mali.

In Mali, several individual observational studies have determined the risk and consequences of malaria in individual pregnant women in areas of high (Bouvier et al., 1997a, Bouvier et al., 1997b) and moderate (Kayentao et al., 2007, Dicko et al., 2003) malaria transmission. However, national estimates of the burden of malaria in pregnancy and its potential impact are lacking. This chapter describes the results of 11 surveys conducted in 9 sites across different malaria transmission settings of the country from 2005 to 2010. Data generated was used to quantify the burden and consequences of malaria in pregnancy by site, region, transmission settings, and at national level through extrapolation.

1.9.3 Chapter 3: Randomised controlled trial of the efficacy and safety of 2 versus 3 doses of IPTp-SP for the prevention of malaria during pregnancy in Mali.

In Mali where resistance to SP is very low (Dicko et al., 2010, Tekete et al., 2009), surveys conducted in 2006-2007 involving 1,696 pregnant women of all parities, showed that placental infection was very common among women who had received the full two-dose

regimen of IPTp (23%; Kayentao et al, unpublished). This is consistent with the finding from an earlier trial showing a significant association between IPTp-SP and the risk of placental infections during the transmission season, especially in women who completed the second dose of IPTp-SP early in the third trimester (Kayentao et al., 2005). This suggested that two doses may provide insufficient protection against reinfections later in the third trimester, a period of rapid *in utero* foetal growth. We therefore conducted a trial comparing the 2-dose IPTp regimen versus three doses using SP, hypothesizing that the third dose may add significant benefit over the 2-dose regimen in preventing placental malaria (primary end point) and other birth outcomes (Diakite et al., 2011).

1.9.4 Chapter 4: A systematic review and meta-analysis of IPTp using 2 versus 3 or more doses of SP on low birth weight in Africa

The results of the above trial (chapter 3) were then pooled with all other similar trials as part of a meta-analysis assessing if three or more doses of IPTp-SP are more effective than the 2-dose regimen and to examine whether this is moderated by SP resistance, HIV status, gravidity or bednet. This meta-analysis was able to identify 6 other clinical trials. The pooled results suggested a marked benefit of adding extra SP doses over the 2-dose regimen in both regions of high and low prevalence of *dhps* K540E mutations (Kayentao et al., 2013). Results gained had guided the World Health Organization to revise IPTp-SP policy (WHO, 2013).

1.9.5 Chapter 5: Parasite clearance following treatment with sulfadoxine-pyrimethamine for intermittent preventive treatment in Burkina-Faso and Mali: 42-day in vivo follow-up study.

This chapter describes the results of a 42-day in-vivo study to determine the parasitological response to a standard single dose of SP when provided as part of the first course of IPTp in asymptomatic parasitaemic pregnant women in one site in Burkina Faso and two sites in Mali. The study was designed to get a better understanding of the efficacy of SP in clearing parasites and the duration of post-treatment prophylaxis to prevent new infections. The study also contains a molecular module to determine the level of SP resistance among the local parasite populations. Monitoring SP resistance was previously mostly based on in-vivo treatment studies in symptomatic children; however, extrapolation from children to asymptomatic pregnant women is difficult because

pregnant women are immunologically different from children. They remain typically asymptomatic when infected and have lower parasites densities than sick children and as a result have typically better treatment responses to antimalarials, including SP (Kalanda et al., 2006). The chapter concludes that SP is very effective in clearing existing infections in Mali and Burkina-Faso.

1.9.6 Chapter 6: Effectiveness of intermittent preventive therapy for the control of malaria in pregnancy in five health districts of Mali

As in chapter 5, this chapter is also part of the studies aimed at obtaining a better understanding of the impact of SP resistance on the effectiveness of IPTp-SP. Chapter 6 describes the result of observational studies conducted in delivery units and the association between the number of doses of SP received by women and clinical parameters. IPTp is shown to be strongly associated with reduced risk of low birth weight and maternal anaemia. Results generated will be added to the existing database on the effectiveness and resistance of IPTp-SP in West–Africa.

1.9.7 Chapter 7: General discussion and conclusion

The final chapter is a synthesised discussion of the main findings in chapter 2 to 6. It highlights new knowledge gained and provides their implication in the control of malaria in pregnancy in Africa. On-going research and future research needs are discussed.

Chapter 2: Mapping the risk of malaria during pregnancy in different transmission settings in Mali

Chapter 2

2 Mapping the risk of malaria during pregnancy in different transmission settings in Mali

2.1 Introduction

Pregnancy associated malaria (PAM) is an overwhelming public health problem in sub-Saharan Africa where 32 million pregnant women are estimated to occur each year (Dellicour et al., 2010). The epidemiology and clinical presentation of PAM depend on the prevailing intensity of malaria transmission (Bardaji et al., 2008, Newman et al., 2003a, Nosten et al., 2004). Disease control strategies in this vulnerable group have focused on a three-pronged approach: intermittent preventive treatment (IPTp) using sulfadoxine-pyrimethamine (SP) in the second and third trimester, use of insecticide treated nets (ITNs) provided at antenatal booking, and effective case management of malarial illness and anaemia (WHO/AFRO, 2004). Deployment of these control strategies is determined by the characteristics of the transmission settings. Effective case management is the primary strategy in areas with low or unstable transmission such as Asia, Latin America and parts of the horn of Africa where most malaria infections eventually result in clinical malaria because of low levels of acquired antimalarial immunity, whilst in higher or stable transmission areas high levels of acquired immunity in adults means most *P. falciparum* infections remain asymptomatic, yet can have harmful consequences for mother and newborn. In these settings, both case management and prevention strategies are used (Bardaji et al., 2008, Menendez et al., 2007, Newman et al., 2003b, Nosten et al., 2004, WHO/AFRO, 2004).

Mali is a landlocked country divided in 8 administrative regions with a wide range of malaria transmission patterns as described earlier (Doumbo O., 1991). Malaria is highly seasonal with most transmission occurring in May-June to October with important differences in intensity and duration of the seasons between the North and the South. The South is characterized by longer rainy seasons and >=1,000 mm of annual rainfall and has a malaria infection prevalence during the peak of the transmission season ranging from 70 to 80% , whereas in the North the seasons are much shorter (2 to 3 months) with an annual rainfall of <300 mm and a peak parasite prevalence below 5% (Doumbo O., 1991, Koita et al., 2012). In addition, some parts of the country have virtually year-round transmission due to irrigation for rice cultivation which helps maintains transmission

outside the rainy seasons (Ceesay et al., 2012, Doumbo O., 1991, Sogoba et al., 2007). The risk and consequences of malaria in pregnancy is likely to vary importantly by administrative region in Mali, yet the control strategy is similar throughout the country.

Using different designs, several individual studies have assessed the burden of PAM in different parts of Mali (Bouvier et al., 1997a, Bouvier et al., 1997b, Diakite et al., 2011, Diallo et al., 2007, Dicko et al., 2003, Kayentao et al., 2005, Kayentao et al., 2007, Maiga et al., 2010). However, national estimates of the absolute burden of PAM are lacking and routine surveillance data of malaria in pregnancy are not available in Mali. National health information systems data suggest that malaria represent 36.5% of consultations in health centres in Mali in 2009, and is the leading cause of mortality and morbidity in children under five and anaemia in pregnant women (Ministère de la Santé, 2009). To allow for efficient allocation of limited resources for the control of malaria in pregnancy, there is a need for estimating the burden of PAM by transmission strata, as suggested by the WHO (WHO, 2000b).

In this chapter we report the burden of malaria in pregnancy in different regions of Mali using data collected from 11 surveys conducted between January 2005 and January 2010. The results of the analysis of the effectiveness of IPTp are reported elsewhere (chapter 6). Here we report the results with regards to peripheral and placental malaria and their associations with birth outcome. The survey data are then used to estimate the national burden of malaria in pregnancy and the potential added benefits from upscaling ITNs and IPTp use (Gamble et al., 2007, Ter Kuile et al., 2007).

2.2 Methods

2.2.1 Study sites and surveys

A total of 11 surveys in 9 sites were conducted between January 2005 and 2010 (Figure 2.1 & Table 2. 1). All the surveys were cross-sectional, used similar methods and had similar objectives to assess the burden of malaria in pregnancy and the effectiveness of IPT and ITNs on birth parameters among women who presented for delivery. The only exception was that during the last 2 surveys conducted from July 2009 to January 2010 in the districts of San and Kita, information on placental and maternal malaria using PCR was available. A detailed description of the study sites is available in chapter 1.

Chapter 2

Table 2. 1: Socio-demographic characteristics

Transmission	Low			Moderate					High			
Sites	Timbuktu (N=213)	Bamako-1 (N=201)	Bamako-2 (N=379)	Koro (N=424)	Djenne (N=424)	Sangha (N=202)	San-1 (N=424)	San-2 (N=471)	Bougouni (N=424)	Fana (N=200)	Kita (N=624)	All 3,986
Regions	North*	Bamako	Bamako	Mopti	Mopti	Mopti	Segou	Segou	Sikasso	Koulikoro	Kayes	
Latitude	16.77	12.65	12.65	13.94	13.9	14.66	13.3	13.3	11.41	12.77	13.05	
Longitude	-3.0	-8.00	-8.00	-3.02	-3.55	-3.31	-4.9	-4.9	-7.48	-6.95	-9.48	
Km from Bamako	907	0	0	722	567	800	423	423	180	127	182	
Study period	Jan05-Mar05	Jan05-Mar05	Mar-09 Nov 09	Sep06-Mar07	Sep06-Mar07	Jun-06 Feb 07	Sep06-Mar07	Jul09-Jan10	Sep06-Mar07	Nov05-Feb06	Jul09-Jan10	
Peak transmission[&]	Sep-Oct	Oct-Nov	Oct-Nov	Sept-Oct	Sept-Oct	Sept-Oct	Sept-Oct	Sept-Oct	Oct-Nov	Oct-Nov	Oct-Nov	
Observed % Mal†	17/163 (10.4)	4/134 (3.0)	0/109 (0.0)	63/179 (35.2)	16/154 (10.4)	7/23 (30.4)	41/147 (27.9)	14/127 (11.0)	29/111 (26.1)	19/102 (18.6)	19/155 (12.3)	229/1,404 (16.3)
Delivery during rainy season‡, n (%)	0 (0.0)	0 (0.0)	270 (71.2)	186 (43.9)	168 (39.6)	155 (77.1)	377 (88.9)	405 (86.0)	272 (64.2)	15 (7.5)	561 (90.3)	2409 (60.4)
Completed primary school, n (%)	52 (24.4)	46 (22.9)	64 (16.9)	100 (23.6)	71 (16.8)	3 (1.5)	83 (19.6)	105 (22.8)	129 (31.1)	40 (20.0)	198 (31.8)	824 (21.4)
Age in years												
Median (range)	23 (15-44)	24 (15-45)	23 (15-45)	25 (15-45)	25 (15-45)	25 (15-45)	25 (15-42)	25 (15-48)	23 (15-46)	23 (15-44)	23 (14-50)	24 (15-42)
<=20 years, n (%)	80 (37.6)	71 (35.3)	161 (42.5)	141 (33.3)	116 (27.4)	30 (19)	122 (28.8)	131 (27.9)	169 (39.9)	77 (38.5)	230 (37.0)	1,347 (34.2)
Married	184 (86.4)	192 (95.5)	349 (93.3)	361 (85.1)	400 (94.3)	190 (94.1)	383 (90.5)	436 (93.4)	384 (90.6)	167 (83.5)	527 (85.0)	3573 (89.8)
Gravidity												
Median (range)	3 (1-11)	3 (1-13)	3 (1-13)	2 (1-10)	3 (1-12)	4 (1-12)	2 (1-9)	2 (1-12)	2(1-11)	3 (1-14)	2 (1-11)	2 (1-10)
G1	66 (31.0)	41 (20.4)	104 (27.4)	117 (27.8)	93 (22.1)	33 (16.3)	100 (23.6)	96 (20.2)	99 (23.5)	43 (21.5)	160 (25.8)	952 (24.0)
G2	38 (17.8)	42 (20.9)	88 (23.2)	59 (14.0)	56 (13.3)	37 (18.3)	85 (20.1)	85 (18.1)	88 (20.9)	39 (19.5)	107 (17.2)	724 (18.2)
G3	26 (12.2)	29 (14.4)	62 (16.4)	55 (13.1)	56 (13.3)	24 (11.9)	61 (14.0)	69 (14.7)	59 (14.0)	19 (9.5)	95 (15.3)	555 (14.0)
G4+	83 (39.0)	89 (44.3)	125 (33)	190 (45.1)	215 (51.2)	108 (53.5)	177 (41.8)	219 (46.7)	176 (41.7)	99 (49.5)	259 (41.7)	1740 (43.8)

Notes: N, sample size; ANC, antenatal clinic; G1, first pregnancy ; G2, second pregnancy; G3, third pregnancy; G4+, four or more pregnancies ;

*The north of Mali includes the regions of Timbuktu, Gao and Kidal

[&]Peak malaria transmission, based on annual rainfall data

†Malaria transmission intensity based on the prevalence of malaria at delivery in women not receiving SP

‡Rainy season defined as the time in months between first and last drops of rain in a given year

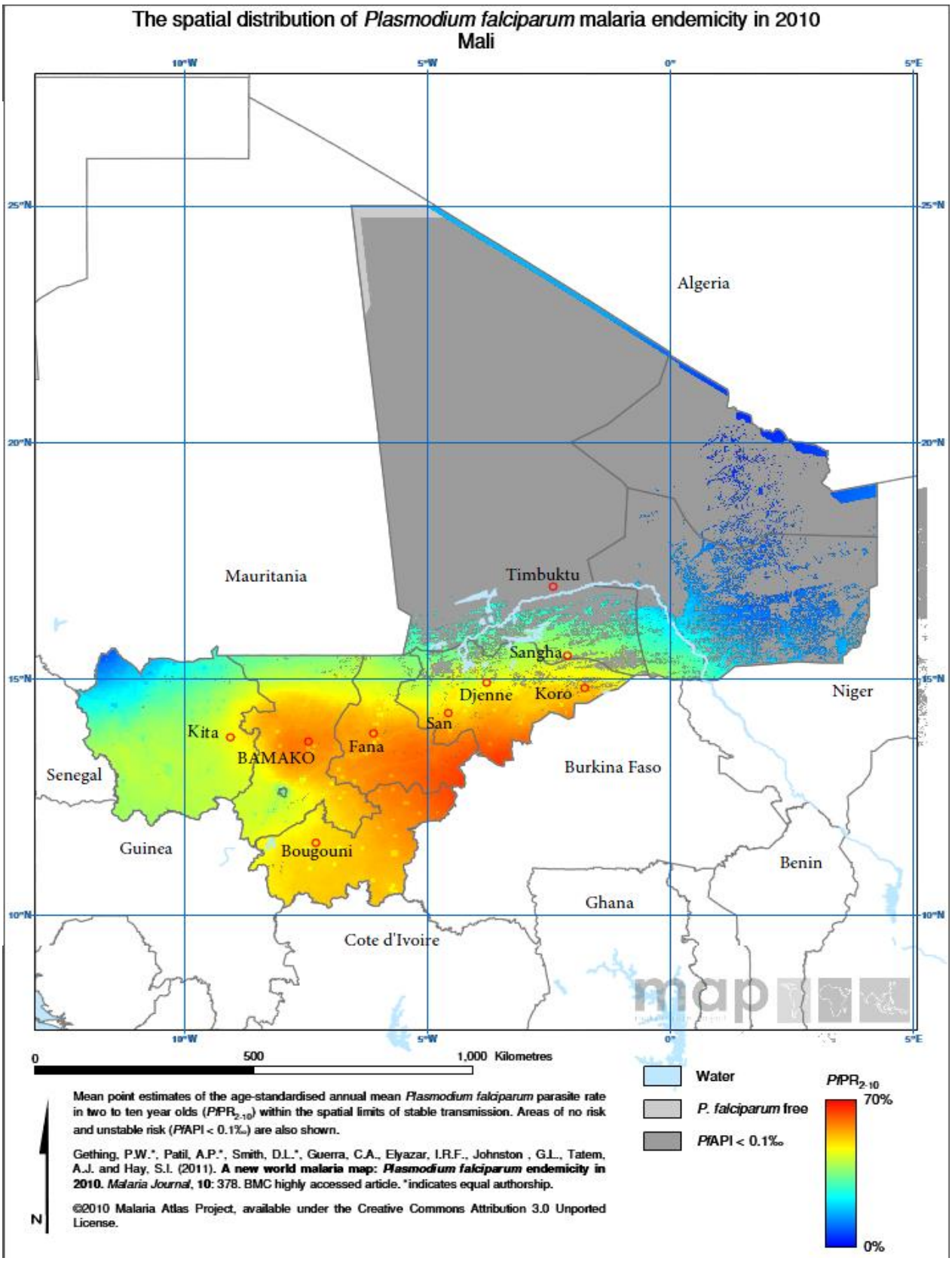


Figure 2.1: Map of Malaria prevalence in Mali with the different study sites
Open circles depict the location of the 9 survey sites.
Source: This map is a product of the MAP project (http://www.map.ox.ac.uk/client_media/pdf/Pf_mean_2010/Pf_mean_2010_MLI.pdf)

2.2.2 Study Procedures

In all the surveys, women of all gravida were screened in the delivery unit of the participating health centre. Before enrolment, women were asked to provide informed consent after the study procedures were explained in their local language (Bambara) and if their age was >=15 years old.

At enrolment, a questionnaire focused on socio-demographic characteristics, history of fever, and use of insecticides treated nets (ITNs), chemoprophylaxis, and intermittent preventive therapy (IPTp). The number of doses of IPTp during pregnancy reported by the woman was verified using antenatal clinic (ANC) cards and records. Details of the number of SP doses was not assessed for the first three surveys in Timbuktu, Bamako and Fana, but any use of IPTp was recorded as >=1 or none. The axillary temperature was measured in degrees Celsius using an digital thermometer and a capillary blood sample taken by finger stick for haemoglobin levels (not in the first three surveys) and malaria thin and thick smears (all surveys). Placental (maternal side) and umbilical cord blood (infant) smears were also taken. In all but 4 surveys (Timbuktu, Bamako [2] and Sangha) dried blood spots were collected for polymerase chain reaction (PCR) for molecular markers of drug resistance (6 surveys) and for detection of malaria (last two surveys). Within 24 hours of delivery, singleton neonates were weighed using identical digital scales. Gestational age was assessed using a standardized Ballard examination (Ballard et al., 1979).

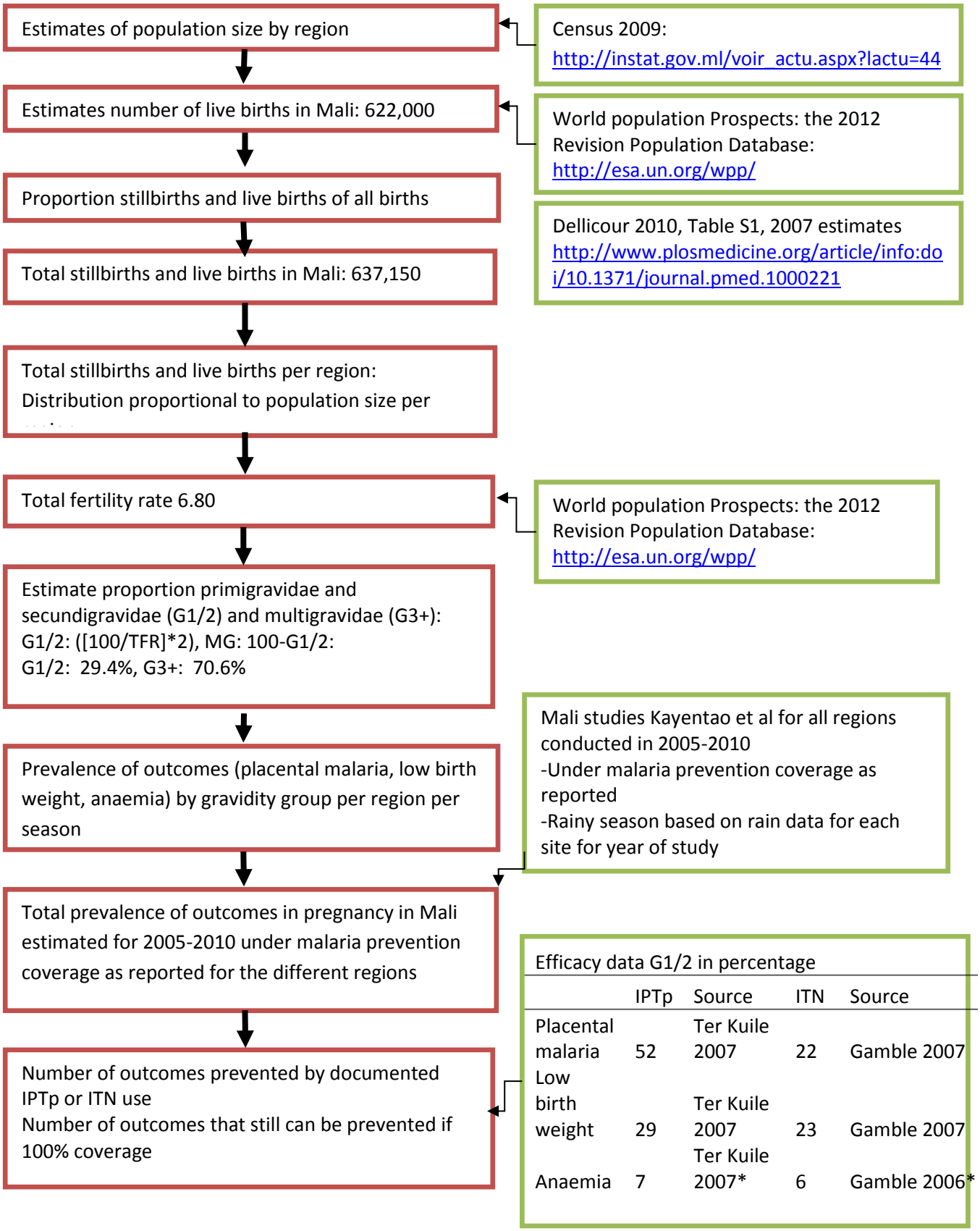
Thick blood smears were stained with Giemsa and examined for malaria parasites. Parasites were counted for against 300 leukocytes, and parasites densities expressed using an assumed leukocytes count of 7,500 /mm^3. Smears were considered negative if no parasites were detected in 100 high power fields. An expert microscopist from the Malaria Research and Training Center (MRTC) read 10% random sample of positive and negative slides for quality control. When >=20% of discrepancy in the results was found between the field microscopists and the expert microscopist when comparing positive vs negative results, all the slides were read again. HemoCue® (Hemoglobin AB, Ångelholm, Sweden) was used to assess haemoglobin levels.

2.2.3 Definitions

LBW was defined as birth weight of <2500 grams in live born singleton births, and prematurity as a gestational age of <37 weeks assessed by Ballard examination. Small for gestational age (SGA) was determined as <10th percentile in birth weight for attained gestational age (Landis et al., 2009). Anaemia was defined as haemoglobin (Hb) levels of <11 g/dL, and severe anaemia as Hb <8 g/dL. The composite LBW-preterm was defined as a new born with either LBW or preterm birth. Women were classified as young if they were ≤20 years old. ITN use was defined as the use of an insecticide treated net the night before the survey as reported by women at delivery.

For the purpose of our analysis we stratified the 9 study sites a *priori* into high (Bougouni, Kita, and Fana), moderate (San, Djenne, Koro, Sangha), and low (Bamako, Timbuktu) transmission settings (Figure 2.1 & Table 2. 1) according to Doumbo et al. (Doumbo O., 1991), (chapter 1). In addition, we used *observed* malaria transmission intensity, which was defined based on the *Plasmodium falciparum* prevalence in the peripheral blood at delivery in women not receiving IPTp, and classified as low if prevalence was <20% and high if ≥20%.

Figure 2.2: Calculation of malaria in pregnancy estimates in Mali



Notes: *Efficacy anaemia by IPTp recalculated from ter Kuile 2007 using data which only compared IPTp vs. no IPTp; efficacy anaemia by ITNs recalculated from Gamble 2006 using data from Browne 1996 and Njagi (Njagi et al., 2003), comparing ITNs vs. no ITNs (Gamble et al., 2006).

Table 2.2:Malaria prevention measures used during pregnancy reported at delivery

Transmission	Low			Moderate					High			All
Sites	Timbuktu (N=213)	Bamako-1 (N=201)	Bamako-2 (N=379)	Koro (N=424)	Djenne (N=424)	Sangha (N=201)	San-1 (N=424)	San-2 (N=471)	Bougouni (N=424)	Fana (N=200)	Kita (N=624)	3986
Bednet use, n (%)	104 (48.8)	126 (62.7)		338(79.7)	416(98.1)		371 (87.5)	454(96.39)	239 (56.4)	150 (75.0)	566(91.0)	2764 (92.5)
ITN last night, n (%)	36 (16.9)	39 (19.4)	306 (81.4)	249 (58.7)	288 (67.9)	109 (63.0)	260 (61.3)	444 (94.5)	152 (35.9)	123 (61.5)	549 (88.3)	2555 (64.7)
IPTp-SP doses, n (%)												
0	165 (78.2)	134 (67.7)	109 (28.8)	180(42.5)	154(36.3)	23 (11.4)	147(34.7)	127(27.1)	111 (26.2)	102 (79.7)	155(25.5)	1307 (34.5)
>=1	46 (21.8)	64 (32.3)	270 (71.2)	244 (57.5)	270 (63.7)	179 (88.6)	277 (65.3)	342 (72.9)	313 (73.8)	26 (20.3)	454 (74.5)	2485 (65.5)
1			111 (29.3)	147(34.7)	119(28.1)	-	186(43.9)	175(37.3)	187(44.1)		132(21.6)	1057 (33.5)
2			159 (42.0)	97(22.9)	151(35.6)	-	91(21.5)	157(33.5)	126 (29.7)		322(53.0)	1103 (35.0)
>=3				0	0	-	0	10 (2.1)	0		0	10 (2.1)

Notes: N, sample size; n, number of events; ITN, insecticide treated nets; IPTp, intermittent preventive treatment; SP, sulfadoxine-pyrimethamine.

2.2.4 Rainfall data

Monthly rainfall data were provided by The Malian Metrological Agency of the Ministry of Equipment and Transports. This was available for the entire year of all study periods for all the sites. Daily rainfall data were not available.

2.2.5 Statistical approach

Data entry was done using access 2000 (Microsoft Office) and data analysis was done using Stata 12.0 (StataCorp LP, Texas) and Microsoft Excel 2010 (Microsoft Office). The prevalence of maternal anaemia, maternal and placental parasitemia, as well as that of LBW, small for gestational age (SGA), preterm delivery, and the composite LBW-preterm was determined per site, region, and transmission settings. Possible risk factors for these outcomes were determined using univariate and multivariate analysis. Crude and adjusted prevalence ratios (aPR) were estimated based on generalized linear models (GLM) using log binomial regression. For non-convergence models, the "binreg" option was used (Cummings, 2009). All predictor variables with a p-value <0.2 in the univariate analysis were included in the multivariable models. However, a *priori* transmission setting was forced into all models because of its epidemiological importance. Other variables considered for inclusion as possible confounders included: age (≤20 versus >20 years), season of delivery (dry versus rainy), gravida group (G1-G2 versus G3+) and ITN (use versus none use). IPTp use was entered as 2 or more doses of IPTp versus women only one course of IPT, or no IPT. Data from the survey of Bamako 2005 was excluded from the analysis of LBW and mean birth weight because this site experienced a calibration issue with their weighing scale which was detected close to the end of the survey. Forest plots and meta-regression was used to determine the association between malaria transmission intensity (defined as the observed prevalence of malaria in the peripheral blood of women at delivery) and the risk of LBW.

2.2.6 Extrapolation to annual national numbers of pregnancies affected and impact of prevention strategies

To obtain estimates of the total number of pregnancies affected annually in Mali, and the potential impact of upscaling the coverage of IPTp and ITNs the results of the survey data were extrapolated to national levels using near identical methods as described previously

to quantify the burden and consequence of malaria in pregnancy in the Democratic Republic of the Congo (Taylor et al., 2011b).

Estimates for the annual number of live and stillbirths were obtained from the United Nations world population database (World-Population-Prospect, 2012) and Dellicour et al. (Dellicour et al., 2010). The national fertility rate (The World Population Prospects 2012 database) (World-Population-Prospect, 2012) was used to obtain the number of births by gravidity. The risk of malaria was assumed to be restricted to the transmission season, which was estimated to last 6, 4 or 3 months in areas with high, moderate and low transmission respectively, except for Bamako which has low transmission, but a 6 months transmission season.

The prevalence of outcomes (malaria, LBW, anaemia) and coverage of prevention strategies (ITNs and IPTp) in the survey were calculated by gravidity group (G1-G2 and G3+), per region (6 region strata where the three northern regions with similar characteristics [Timbuktu, Gao and Kidal] were pooled into the single region of Timbuktu) and eventually per transmission strata (*a priori* set as high, moderate, low) (Table 2. 1).

For the site of Timbuktu rainy season burden data was not available. We estimated the risk ratio of rainy to dry season for placental malaria among primi-and secundigravida for all other sites; the pooled risk was 1.7; 95% CI, 1.2-2.6, I-squared 51%, and used this to estimate the rainy season prevalence of placental malaria in Timbuktu. For multigravida, we extrapolated the measured prevalence for the dry season to the rainy season because it was perceived as high for the area.

The observed prevalence estimates for placental malaria, LBW, and anaemia in our surveys, combined with the absolute number of pregnancies at risk were then used to estimate the total number of births resulting in placental malaria, LBW, and anaemia in each region, transmission strata and nationally.

The potential impact of successful malaria control in pregnancy was estimated by using the summary protective efficacy (PE) of ITNs and IPTp-SP obtained from previous meta-analyses of randomized controlled trials comparing IPTp-SP against placebo or passive case detection (case-management). The published protective efficacy estimates

for ITNs and IPTp-SP among first and second pregnancies are 23% and 29% for LBW, 52% and 22% for placental malaria and 7% and 6% for anaemia at delivery (Hb<10 or 11 g/dL), respectively (Figure 2.2).

The reduction in the risk of each outcome (event) from IPTp or ITNs under the observed coverage of 2-dose IPTp or ITNs in G1-G2 (first and second pregnancies) in the 11 surveys was then calculated as 1/(1-(coverage% * PE%)), where the coverage% is the observed coverage of 2-dose IPTp or ITNs in the specific region and PE% is the % protective efficacy observed in trials. The number of events prevented under the observed coverage of the intervention was then calculated as the number of events if there was no IPTp or ITN (0% coverage), minus the observed number of events. The potential number of events that could be prevented if all women used IPTp was calculated as the number of events under 0% coverage times the protective efficacy estimated from trials; the potential number that could be prevented by increasing the coverage from the observed value to universal coverage was estimated by deducting the observed events from this figure (Taylor et al., 2011b).

For example, for the effect of 2-dose IPTp on LBW in primi,- and secundigravida in the Kayes region, the coverage of 2-dose IPTp was 55.3% in G1-G2. The estimated % reduction in the LBW with 55.3% 2-dose of IPTp coverage was calculated as 0.553 * 29% = 16.0%, where 29% is the PE value obtained from the meta-analysis for the effect of 2-dose IPTp on LBW. To calculate the annual number of LBW events that would have occurred in the absence of a 2-dose IPTp strategy, the estimated LBW events based on the observed risk of LBW and the observed coverage of 2-dose IPTp or ITNs was divided by a factor of 1-(coverage % x PE%), where coverage% is the observed coverage of the intervention in the region, and PE% is the value obtained from the meta-analysis of trials. For example in Kayes, the average risk of LBW was 20.25% in G1-G2, and the annual number of births was 25,776, giving 5,220 LBW events per year; the events that would have occurred without any IPTp strategy was: 5,220 / (1-(55.3% x 29.0%)) = 6,217. Thus the 55.3% coverage prevented 6,217-5,220 = 997 LBW events annually. The additional number of LBW that could be prevented by increasing the coverage to 100% (universal coverage) was calculated as the number of LBW events that would potentially be

prevented with 100% coverage of 2-dose IPTp (29% of 6,217 = 1,803) minus the number of events prevented under the current coverage : 1,803-997=806.

2.3 Ethical statement

The institutional review boards (IRB) of the Faculty of Medicine, Pharmacy, and Dentistry of Mali (FMPOS) (all surveys), the Centers of Diseases Control and Prevention (CDC) (middle 4 of 5 surveys), and the Liverpool School or Tropical Medicine (LSTM) (last 2 surveys) approved the study protocols.

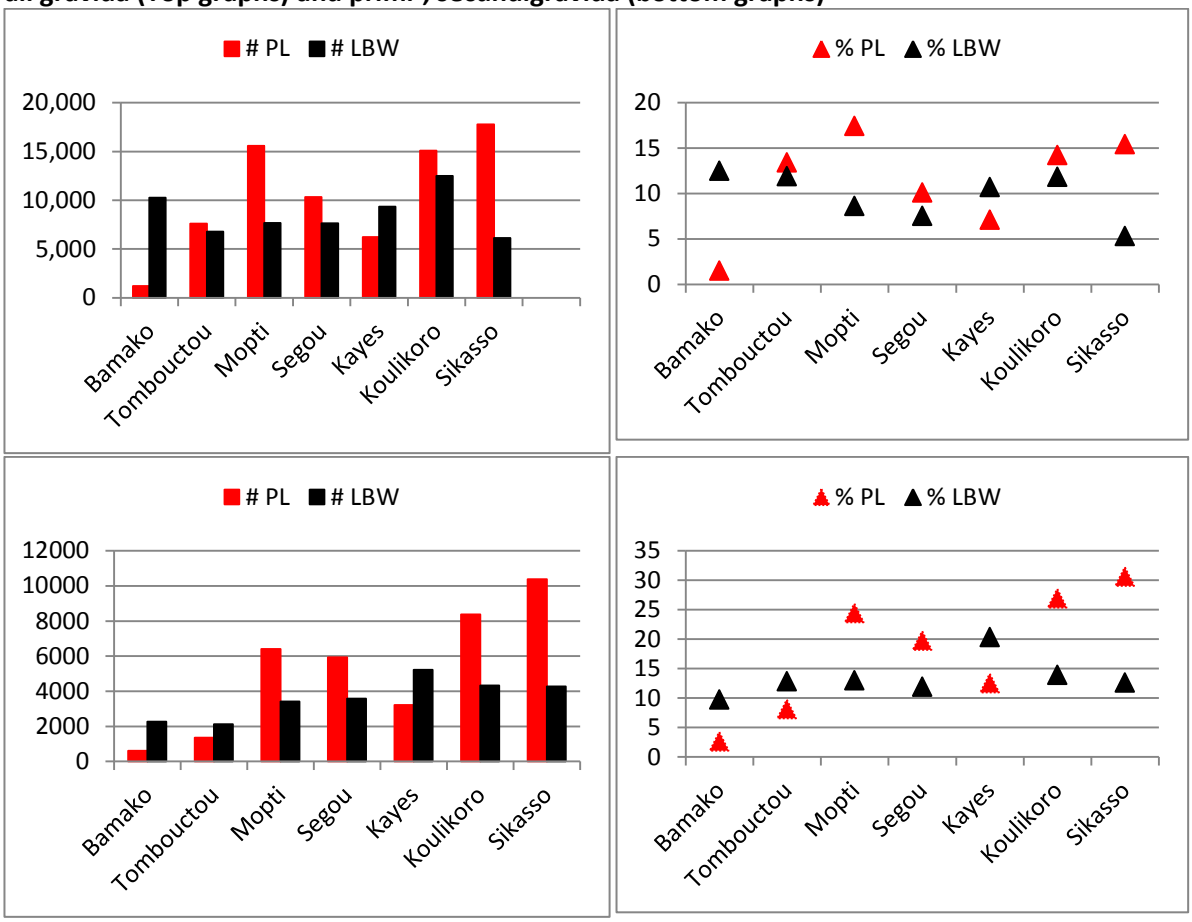
2.4 Results

Characteristics: From 2005 to 2010, 11 surveys were conducted in 9 sites involving a total of 3,982 deliveries of which 793 (19.9%), 1,945 (48.8%), and 1,248 (31.3%) occurred in the low, moderate, and high malaria transmission areas, respectively (Table 2. 1). The median (range) duration of each survey was 7 (1-12) months and most of the deliveries occurred during the rainy season (60.4 %). The median (range) ITN use was 61.5% (16.9-94.5) varying from 16.9% in Timbuktu in 2005 to 94.5% in San in 2009 (Table 2.2). Among the 7 surveys which collected information on the number of IPTp-SP doses received, the median (range) coverage of >=2 doses was 35.4% (21.5-53.2) and highest in Kita (2009) (53.2%) and lowest in San (2006) (21.5%) and Koro (2006) (23%) (Table 2.2). Of the singleton deliveries, 5.8% resulted in stillbirths ranging from 12.5% and 8.9% in Djenne and Koro to 1.5% and 1.1% in Sangha and Bamako-2009. More than half of the women were anaemic (56.5 %) and 10.8% had moderate-severe anaemia.

Malaria: Overall, malaria parasitemia in the peripheral and placental blood was found in 15.2% and 13.2% of women, respectively. The highest for both was observed in Koro, a moderate transmission area, and the lowest in Bamako, a low transmission area (Table 2.3 & Figure 2.3). Risk factors for both maternal and placental parasitemia (Table 2.4) by both univariate and multivariate analysis, were living in areas defined (*a priori*) as moderate transmission areas, being primi-or secundigravidae, and delivering during the rainy season, not using an ITN, and being single or divorced (not married). Young women were more likely to have maternal and placental parasitemia in univariate analysis, but not in multivariate analysis. The receipt of two doses of IPTp-SP was associated with a

32% and 33% reduction of both maternal peripheral and placental parasitemia (Table 2.4), respectively in univariate analysis and 22% and 21% in multivariate analysis, respectively.

Figure 2.3: Number (left graphs) and prevalence (right graphs) of outcomes per region among all gravida (Top graphs) and primi-, secundigravida (bottom graphs)



Notes: PL, placental malaria infection; LBW, low birth weight.
# indicates absolute number; % indicates prevalence of outcomes

Table 2.3: Clinical and biological outcomes

Transmission	Low			Moderate					High			All
Sites	Timbuktu	Bamako-1	Bamako-2	Koro	Djenne	Sangha	San-1	San-2	Bougouni	Fana	Kita	
Birth Outcome												
Live born, n (%)	195 (93.3)	189 (96.4)	359 (98.4)	357 (86.2)	356 (87.0)	193 (98.5)	399 (95.5)	450 (95.5)	407 (96.9)	174 (91.6)	578 (92.9)	3657 (93.5)
Miscarriage, n (%)	4 (1.9)	3 (1.5)	2 (0.5)	20 (4.8)	2 (0.5)	0 (0)	2 (0.5)	2 (0.4)	1 (0.2)	3 (1.6)	5 (0.8)	44 (1.1)
Stillbirth, n (%)	10 (4.8)	4 (2.0)	4 (1.1)	37 (8.9)	51 (12.5)	3 (1.5)	17 (4.1)	19 (4.0)	12 (2.9)	13 (6.8)	39 (6.3)	209 (5.8)
Birth weight												
Grams, Mean (SD)	3004 (536)	2919 (500)	2957 (517)	3183 (562)	3151 (499)	2820 (405)	3089 (518)	3042 (445)	3130 (519)	2913 (487)	3027 (518)	3044 (514)
LBW, n (%)	23 (12.2)	41 (21.7)*	41 (11.4)	27 (7.7)	23 (6.5)	34 (17.6)	29 (7.3)	40 (9.2)	28 (6.9)	21 (12.1)	58 (10.4)	365 (10.1)
Gestational age												
SGA	61 (33.3)	35 (19.0)	**	68 (19.5)	96 (9.7)	114 (60.0)	125 (31.4)	152 (35.4)	121 (30.0)	40 (23.4)	183 (33.0)	995 (31.0)
Weeks, Mean (SD)	41 (3)	37 (3)	**	39 (1)	40 (1)	38 (1)	41 (1)	40 (1)	40 (1)	39 (3)	39 (1)	39 (2)
Preterm (<37wks), n (%)	17 (9.3)	65 (35.3)	11 (2.9)	10 (2.9)	9 (2.6)	8 (4.2)	8 (2.0)	1 (0.2)	7 (1.7)	19 (11.1)	12 (2.2)	167 (4.6)
Preterm-LBW	31 (16.9)	82 (44,6)	43 (11.9)	27 (7.8)	24 (6.9)	37 (19.3)	32 (8.0)	40 (9.3)	26 (6.5)	29 (17.0)	59 (10.6)	430 (12.0)
Haemoglobin, g/dL												
Mean (SD)	-	-	-	10.6(2.2)	10.4(2.1)	-	10.6(2.0)	10.5(1.85)	10.7(2.0)	-	10.6(2.0)	10.5 (2.0)
Hb<11, n (%)	-	-	-	239(56.4)	239 (56.4)	-	286 (67.5	266(58.3)	210(49.5)	-	218(50.8)	1458 (56.5)
Hb<8, n (%)	-	-	-	46 (10.9)	54(12.7)	-	55 (13.0)	36(7.9)	45(10.6)	-	42(9.8)	278 (10.8)
Placental Malaria, n (%)	24 (11.3)	4 (2.0)	6 (1.6)	126 (31.0)	49 (11.7)	20 (10.1)	99 (23.4)	31 (6.7)	88 (20.8)	20 (10.0)	57 (9.4)	524 (13.3)
Maternal Malaria, n (%)	19 (9.1)	5 (2.5)	9 (2.4)	126 (29.8)	48(11.5)	23 (11.5)	117(27.6)	44(9.5)	121(28.6)	32 (16.1)	59 (9.6)	603 (15.3)

Notes: SD, standard deviation; LBW, low birth weight; SGA, small for gestational age; g/dL, grams per decilitre; Hb, haemoglobin;

*In Bamako-1, the high prevalence of LBW resulted from a calibration problem of the weighing scale (resulting in a systematic underestimation of the true weight of the baby). This was only discovered towards the end the survey

**Gestational age was not correctly recorded for Bamako 2009 and Ballard measurement was not used.

Table 2.4:Bivariate and multivariate log binomial regression models of the risk of maternal (upper) and placental (lower) parasitemia

				Maternal Malaria			
		No. of women	% with malaria	PR (95% CI)	P-value	APR (95% CI)	P-value
Transmission	Low	789	4.2	0.24 (0.17-0.34)	<0.001	0.17 (0.09-0.33)	<0.001
	Moderate	1,929	18.6	1.10 (0.93-1.27)	0.294	1.27 (1.08-1.49)	0.004
	High	1,235	17,1	1		1	
Marital status	Married	3,545	14.2	1		1	
	Not married	402	24.4	1.72 (1.42-2.08)	<0.001	1.27 (1.03-1.56)	0.022
Age	>20	2,568	13.4	1		1	
	<=20	1,341	18.9	1.41 (1.21-1.63)	<0.001	0.85 (0.69-1.03)	0.100
Season	Rainy	2,880	18.2	2.58 (2.05-3.25)	<0.001	2.60 (1.90-3.56)	<0.001
	Dry	1,073	7.1	1			
Gravidity	G1-G2	1,667	20.4	1.78 (1.53-2.06)	<0.001	1.87 (1.54-2.30)	<0.001
	G3+	2,286	11.5	1			
ITN use	Yes	2,534	13.1	0.69 (0.59-0.80)	<0.001	0.59 (0.51-0.69)	<0.001
	No	1,416	19.1	1			
IPTp doses*	0	982	18.5	1			
	1	1,052	18.8	1.02 (0.85-1.22)	0.868	1.00 (0.84-1.18)	0.961
	2	1,108	12.9	0.68 (0.57-0.85)	<0.001	0.78 (0.64-0.95)	0.015
				Placental malaria			
		No. of women	% with malaria	PR (95% CI)	P-value	APR (95% CI)	P-value
Transmission	Low	789	4.3	0.33 (0.23-0.48)	<0.001	0.15 (0.07-0.33)	<0.001
	Moderate	1,911	17.0	1.31 (1.1-1.56)	0.003	1.49 (1.24-1.80)	<0.001
	High	1,225	13.0	1			
Marital status	Married	3,523	12.2	1			
	Not married	396	22.5	1.85 (1.51-2.27)	<0.001	1.32 (1.06-1.65)	0.013
Age	>20	2,553	11.5	1			
	<=20	1,328	16.8	1.46 (1.25-1.72)	<0.001	0.90 (0.72-1.12)	0.348
Season	Rainy	2,858	15.7	2.39 (1.88-3.04)	<0.001	2.66(1.89-3.75)	<0.001
	Dry	1,067	6.6	1			
Gravidity	G1-G2	1,648	17.8	1.80 (1.53-2.12)	<0.001	1.83 (1.46-2.30)	<0.001
	G3+	2,277	9.9	1			
ITN use	Yes	2,516	11.5	0.70 (0.60-0.82)	<0.001	0.62 (0.53-0.74)	<0.001
	No	1,406	16.4	1			
IPTp doses*	0	957	16.9	1			
	1	1,049	15.4	0.91 (0.75-1.11)	0.367	0.92 (0.76-1.11)	0.365
	2	1,104	11.4	0.67 (0.54-0.84)	<0.001	0.79 (0.63-0.97)	0.027

Notes: No, sample size; PR, prevalence ratio; APR, adjusted prevalence ratio; G1-2, 1st or 2nd pregnancy ; G3+, >= 3rd pregnancy; IPTp-SP, intermittent preventive treatment with sulfadoxine-pyrimethamine
*Not assessed by dose in 4 surveys (Bamako 2005, Timbuktu, Sangha, and Fana).

Table 2.5: Bivariate and multivariate log binomial regression models of the risk of low birth weight* (upper) and the composite of low birth weight or preterm birth (lower)*

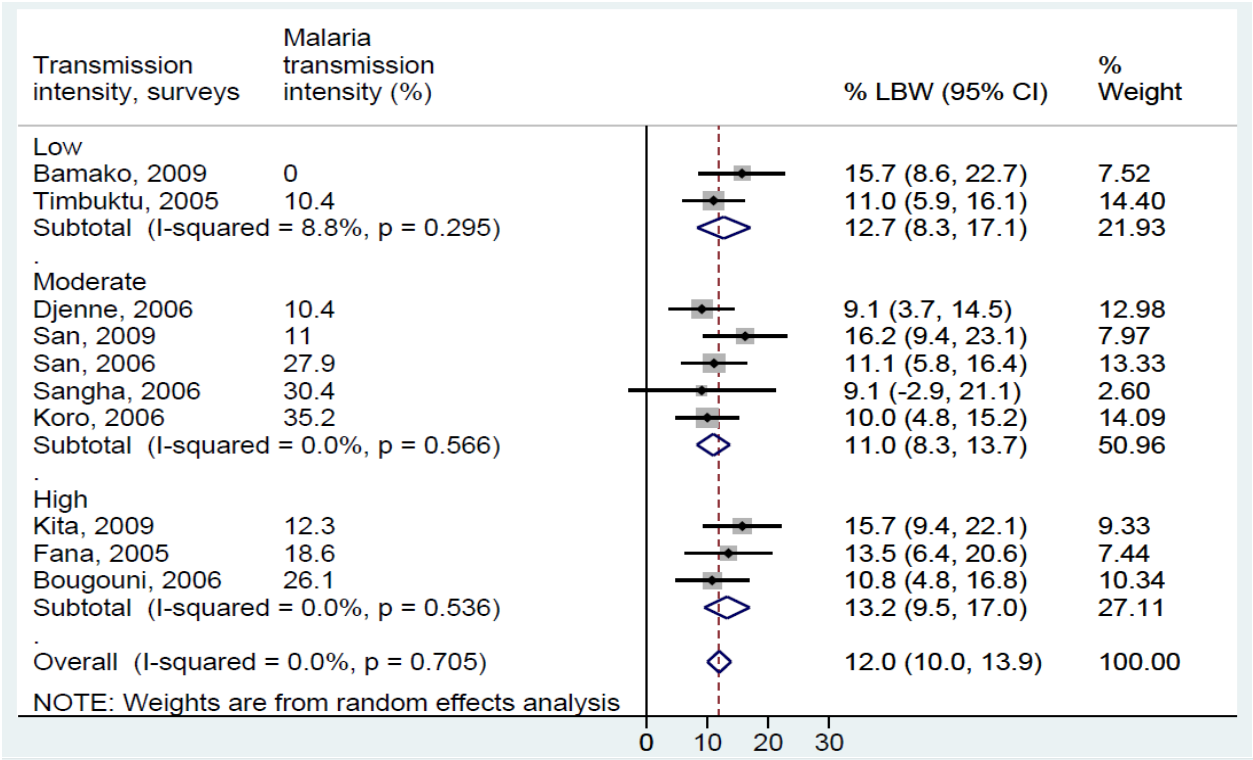
				Low Birth Weight			
		No. of women	% with LBW*	PR (95% CI)	P-value	APR (95% CI)	P-value
Transmission	Low	550	11.8	1.26 (0.94-1.68)	0.122	1.26 (0.89-1.79)	0.196
	Moderate	1,731	8.8	0.94 (0.74-1.19)	0.612	0.87 (0.67-1.13)	0.303
	High	1,139	9.4	1			
Marital status	Married	3,058	8.7	1			
	Not married	355	16.3	1.87 (1.44-2.43)	<0.001	1.38 (1.00-1.90)	0.049
Age	>20	2,217	7.2	1			
	<=20	1,159	13.8	1.91 (1.55-2.35)	<0.001	1.59 (1.13-2.24)	0.009
Season	Rainy	2,651	9.2	0.87 (0.69-1.11)	0.267		
	Dry	769	10.5	1			
Gravidity	G1-G2	1,444	13.0	1.85 (1.50-2.28)	<0.001	1.22 (0.85-1.74)	0.275
	G3+	1,973	7.0	1			
ITN use	Yes	2,292	9.2	0.91 (0.73-1.13)	0.389		
	No	1,121	10.1	1			
IPTp doses**	0	817	12.6	1			
	1	989	7.1	0.56 (0.42-0.75)	<0.001	0.59 (0.45-0.80)	<0.001
	2	1,053	7.0	0.56 (0.42-0.74)	<0.001	0.60 (0.45-0.80)	0.001
Placental Malaria	Positive	463	12.1	1.32 (1.00-1.73)	0.044	1.28 (0.94-1.73)	0.118
	Negative	2,926	9.2	1			
				Low birth weight or preterm*			
		No. of women	% with LBW/PT	PR (95% CI)	P-value	APR (95% CI)	P-value
Transmission	Low	546	13.9	1.35 (1.03-1.76)	0.031	1.21(0.84-1.73)	0.301
	Moderate	1,719	9.4	0.91 (0.73-1.14)	0.423	0.88 (0.68-1.15)	0.362
	High	1,132	10.3	1			
Marital status	Married	3,040	9.5	1			
	Not married	350	18.9	1.98 (1.56-2.53)	<0.001	1.42 (1.04-1.94)	0.028
Age	>20	2,202	8.1	1			
	<=20	1,151	14.9	1.85 (1.52-2.25)	<0.001	1.42 (1.01-1.99)	0.040
Season	Rainy	2,637	9.8	0.76 (0.61-0.94)	0.012	0.99 (0.71-1.38)	0.964
	Dry	760	12.9	1			
Gravidity	G1-G2	1,431	7.6	1.90 (1.55-2.32)	<0.001	1.34 (0.95-1.89)	0.096
	G3+	1,963	14.4	1			
ITN use	Yes	2,278	9.6	0.78 (0.65-0.98)	0.029	1.16 (0.90-1.51)	0.254
	No	1,112	12.1	1			
IPTp doses**	0	811	13.3	1			
	1	982	7.3	0.55 (0.42-0.73)	<0.001	0.58 (0.44-0.78)	<0.001
	2	1,050	7.2	0.54 (0.11-0.16)	<0.001	0.58 (0.43-0.77)	<0.001
Placental malaria	Positive	461	12.8	1.26 (0.97-1.64)	0.083	1.27 (0.93-1.72)	0.131
	Negative	2,906	10.2	1			

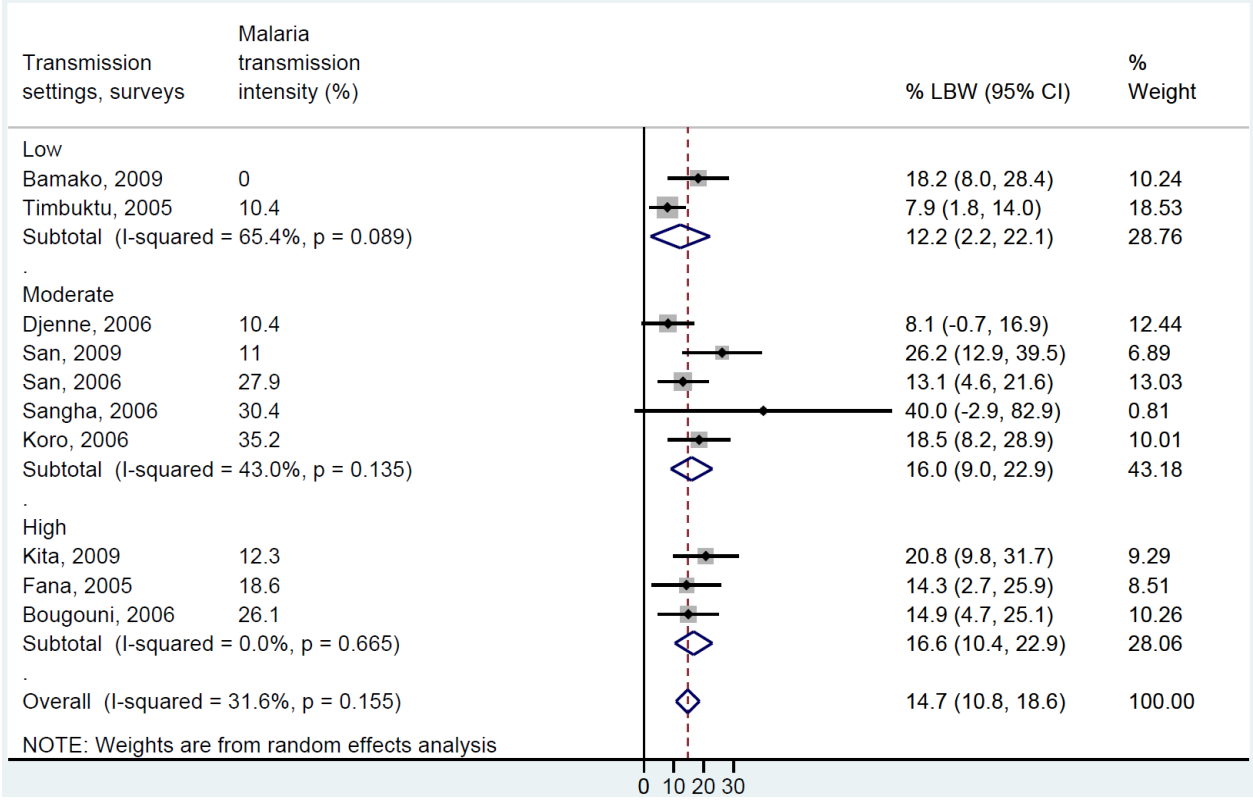
Notes: No, sample size; LBW, low birth weight; PT, preterm, PR, prevalence ratio; APR, adjusted prevalence ratio; G1-2, 1st or 2nd pregnancy ; G3+, >=3rd pregnancy; IPTp-SP, intermittent preventive treatment with sulfadoxine-pyrimethamine; *Bamako 2005 survey was excluded due to calibration problem of the weighing scale (tempted to lower baby's weight); **Not assessed by dose in 4 surveys (Bamako 2005, Timbuktu, Sangha, and Fana).

LBW, SGA, preterm delivery, and LBW-preterm birth: Among the livebirths, the prevalence of LBW was 10.1% and highest in Bamako-2005 (21.7%) and lowest in Djenne (6.5%). The prevalence of small for gestational age (SGA) was 31.0% overall with the highest in Sangha (60.0%) and the lowest in Bamako 2005 (19.0%). The overall prevalence of preterm delivery (PTD) and the composite LBW-preterm was 4.6% and 12.3%, respectively. Risk factors for LBW in multi-variate models included not being married and young age (Table 2.5). Use of IPTp was strongly associated with a lower risk of LBWs in both univariate and multivariate analysis. Placental malaria was associated with an increased risk of LBW in univariate, but not in multivariate analysis. Transmission setting was not associated with the risk of LBW (Table 2.5). The same associations were found for the composite of LBW-preterm births (Table 2.5).

Using a meta-analysis approach, the prevalence of LBW assessed in women not receiving SP was similar across levels of transmission intensity, defined a *priori* as low, moderate, and high or based on the observed prevalence of maternal peripheral malaria (<20% versus >=20%) in women not receiving SP (Figure 2.4 & Figure 2.5). The same pattern was seen when stratified by gravidity.

Figure 2.4: Prevalence of low birth weight by transmission intensity defined a priori as low, moderate and high

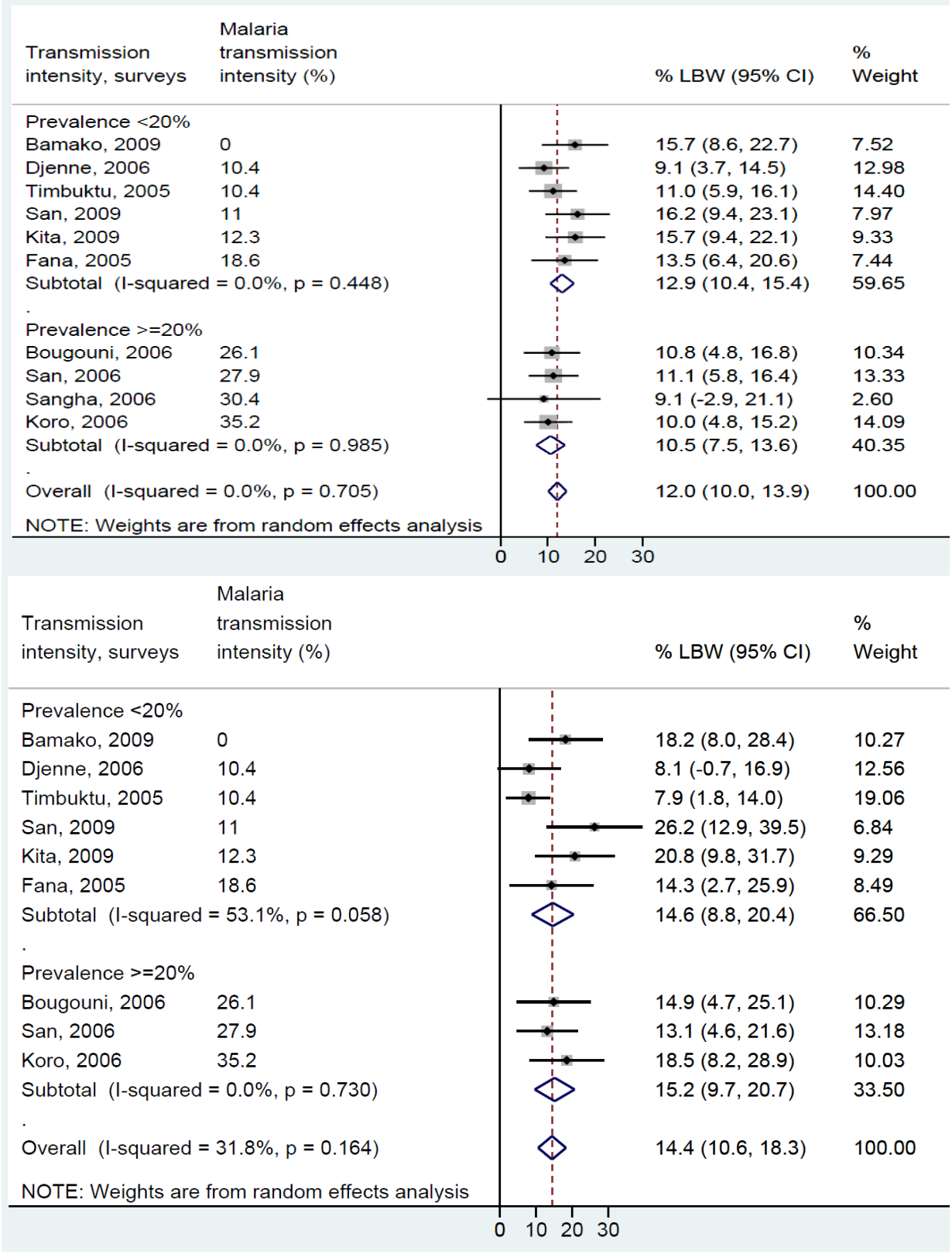




Notes: LBW, low birth weight

Prevalence of low birth weight in all gravida (Top graph) and primi-secundigravida (Bottom graph) by transmission intensity defined a *priori* as low, moderate, and high

Figure 2.5: Prevalence of LBW by observed transmission intensity defined as <20% and >= 20% in women without SP



Notes: LBW, low birth weight;
Prevalence of low birth weight in all gravida (Top graph) and primi-secundigravida (Bottom graph) by observed transmission intensity defined as <20% or >=20% in women without SP.

When adjusted for surveys, marital status, age of women and gravidity, placental malaria was associated with an increased risk of LBW, SGA, the composite LBW or SGA or PTD, any anaemia and moderate to severe anaemia (Table 2.6); and a decrease in mean haemoglobin (Table 2.7). Women with placental infection were more likely to deliver preterm babies, although statistical significance was not reached. Overall, there was a decrease in mean birth weight among women with placental infection, but significant level was reached only for primi,-secundigravida (Table 2.7).

Table 2.6:Association between placental malaria and the risk of LBW, small for gestational age, preterm delivery, and their composite, maternal anaemia by gravidity in univariate and multivariate analysis

	LBW*				SGA**				Preterm			
	PR	P-value	aPR	P-value	PR	P-value	aPR	P-value	PR	P-value	aPR	P-value
All	1.32 (1.01-1.73)	0.044	1.46(1.10-1.94)	0.009	1.16 (1.02-1.33)	0.027	1.25 (1.09-1.44)	0.001	0.86 (0.54-1.38)	0.541	1.29 (0.81-2.05)	0.277
G1-G2	1.48 (1.09-2.01)	0.011	1.74(1.27-2.42)	0.001	1.14 (0.97-1.33)	0.120	1.32 (1.11-1.57)	0.002	0.99 (0.58-1.70)	0.976	1.32 (0.78-2.26)	0.308
G3+	0.67 (0.36-1.26)	0.219	0.77 (0.41-1.45)	0.412	1.00 (0.79-1.29)	0.954	1.09 (0.86-1.39)	0.486	0.49 (0.18-1.32)	0.159	0.96 (0.34-2.68)	0.934
	LBW or SGA or PTD				**Any anaemia (Hb<11 g/dL)**				**Moderate to severe anaemia (Hb<8 g/dL)**			
	PR	P-value	aPR	P-value	PR	P-value	aPR	P-value	PR	P-value	aPR	P-value
All	1.25 (1.10-1.42)	0.001	1.27 (1.12-1.45)	<0.001	1.33 (1.24-1.43)	<0.001	1.31 (1.22-1.41)	<0.001	2.01 (1.58-2.56)	<0.001	1.91 (1.48-2.46)	<0.001
G1-G2	1.29 (1.11-1.50)	0.001	1.27 (1.01-1.50)	0.003	1.32 (1.20-1.46)	<0.001	1.31 (1.19-1.44)	<0.001	2.07 (1.49-2.86)	<0.001	1.76 (1.25-2.46)	<0.001
G3+	1.03 (0.81-1.31)	0.800	1.06 (0.84-1.34)	0.627	1.29 (1.16-1.45)	<0.001	1.30 (1.16-1.47)	<0.001	1.81 (1.25-2.65)	0.002	2.05 (1.46-2.89)	<0.001

Notes: LBW, low birth weight; SGA, small for gestational age; PR, prevalence ratio; aPR, adjusted prevalence ratio (adjustment a *priori* for survey, marital status, age (>20 versus <=20 years old) and gravidity; Hb, haemoglobin; G1-G2, first and second pregnancies; G3+, third or more pregnancy.
*Bamako 2005 was excluded from this analysis.
**Bamako 2009 was excluded from this analysis.

Table 2.7: Difference in mean birth weight and mean haemoglobin among women with placental malaria versus no placental malaria by gravidity in univariate and multivariate analysis

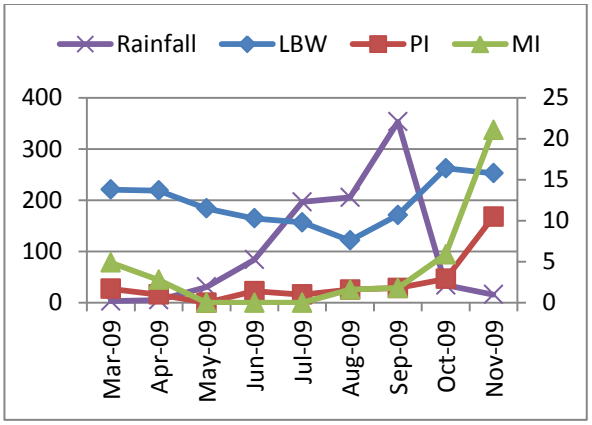
	Mean difference in birth weight*				Mean difference in haemoglobin			
	Univariate	P-value	Multivariate**	P-value	Univariate	P-value	Multivariate**	P-value
All	-28 (-78, 22)	0.270	-37 (-87, 13)	0.141	-0.83 (-1, -63)	<0.001	-0.76 (-0.97, -0.54)	<0.001
G1-G2	-35 (-102, 32)	0.306	-86 (-155, -19)	0.013	-0.93 (-1, -0.64)	<0.001	-0.82 (-1.11, -0.53)	<0.001
G3+	49 (-24, 123)	0.187	20 (-54, 94)	0.593	-0.63 (-0.93, -0.33)	<0.001	-0.64 (-0.94, -0.34)	<0.001

Notes: G1-G2, first and second pregnancies; G3+, third or more pregnancy.
Mean differences represent the mean value among women with placental malaria minus the mean value among women without placental malaria.
*Bamako 2005 was excluded from this analysis.
** Multivariate analysis adjusted *a priori* for survey, marital status, age and gravidity.

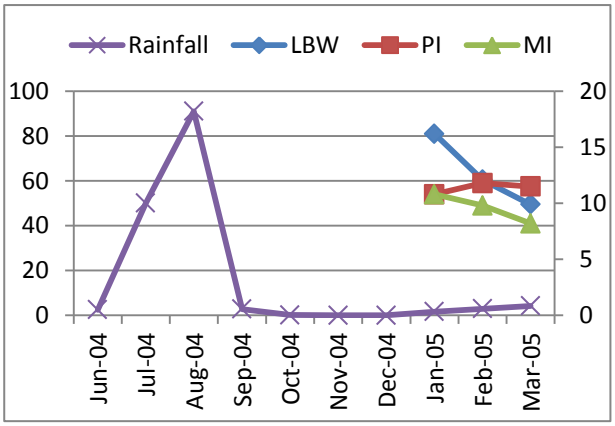
Seasonal trends: Some of the surveys that were conducted during the rains and that continued for several months afterwards showed that the prevalence of maternal and placental malaria typically peaked towards the end of the rainy season or within 1 month after the rains had subsided (Figure 2.6). A similar trend was not apparent for LBW except for the sites of San 2009 and Kita, but overall difference in the prevalence of LBW by month were much smaller. For North Mali data was collected only during the dry season and the relationship to rainfall could not be assessed.

Figure 2.6: Rainfall data and prevalence of outcomes determined during the surveys for each site.

Low transmission settings

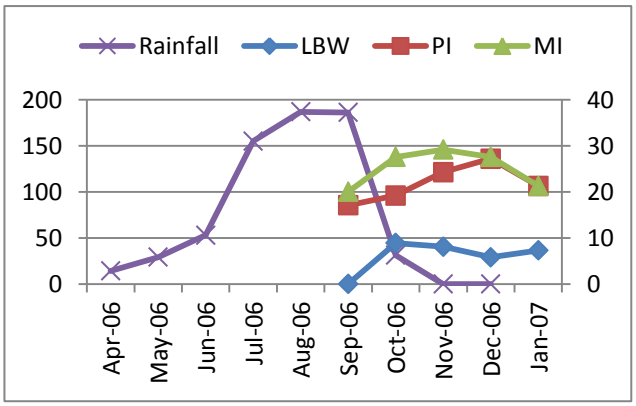


Bamako, 2009

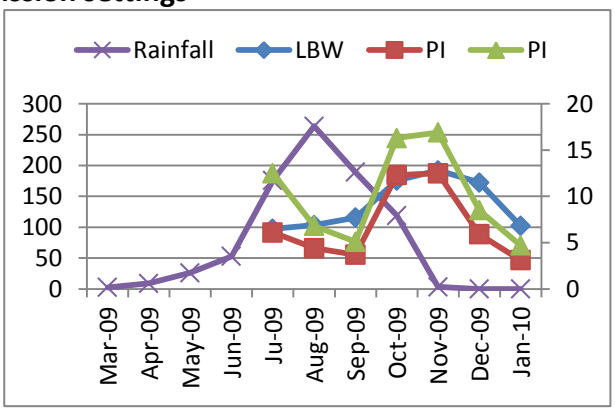


Timbuktu & Gao & Kidal, Timbuktu 2005

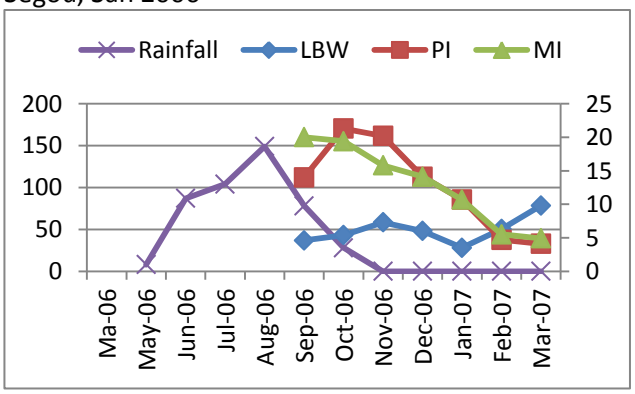
Moderate transmission settings



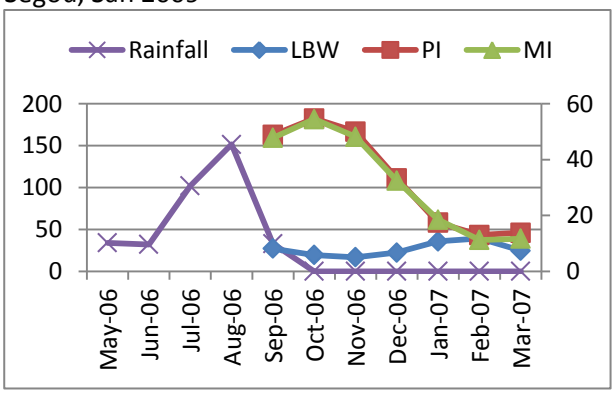
Ségou, San 2006



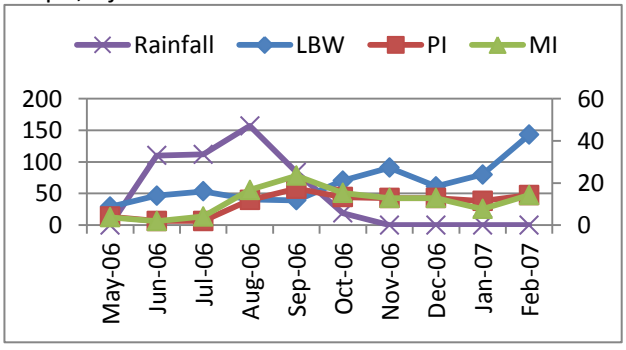
Ségou, San 2009



Mopti, Djenne 2006-200

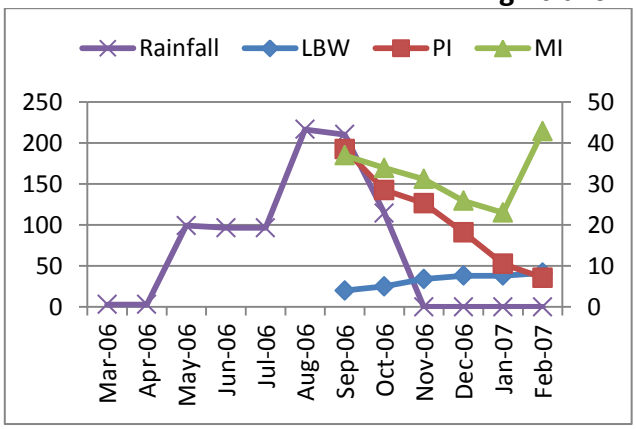


Mopti, Koro 2006-2007

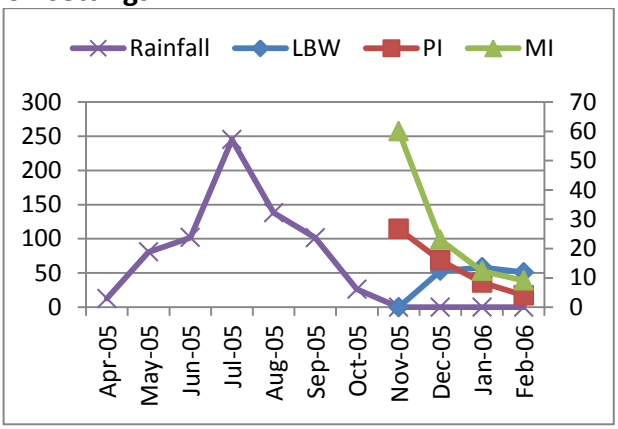


Mopti, Sangha, 2006-2007

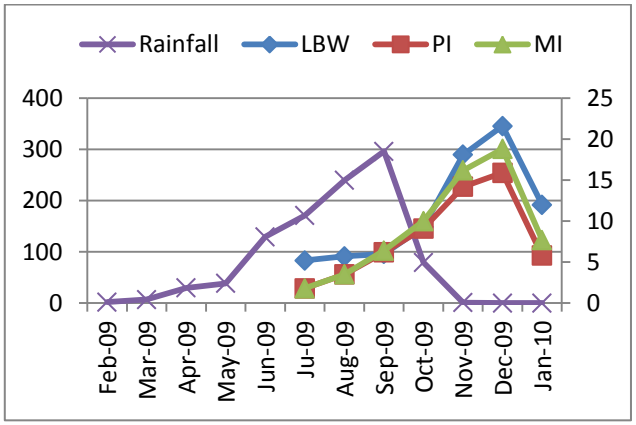
High transmission settings



Sikasso, Bougouni 2006-2007



Koulikoro, Fana 2005-2006



Kayes, Kita 2009

Notes: LBW, Low birth weight; PI, placental malaria infection; MI, maternal malaria infection; Rainfall in the left Y-axis is in millimeters; prevalence of outcomes in the right Y-axis.

A three-month moving average was used to estimate the prevalence of LBW, maternal and placental malaria. Malaria transmission is depicted by the average monthly rainfall in the study locations during the study period.

Chapter 2

Extrapolation of the prevalence of placental malaria parasitemia, low birth weight, and anaemia to the total population:

In 2009, an estimated 637,150 births occurred in Mali (Table 2.8). Based on the average prevalence of placental malaria of 11.6% (95% confidence interval [CI], 11.5-11.7) observed across the 11 surveys in this study, 73,775 (95% CI, 73,274-74,276) births may be affected by placental malaria infection every year in Mali. The corresponding figures for primi,- and secundigravidae were 36,248/187,397 (19.3%) were affected by placental malaria. Universal coverage with 2-dose IPTp-SP among this gravidity group has the potential to reduce placental malaria by 52% from 36,248 birth annually under the current coverage (30.4%) to 20,510 births annually and universal coverage of ITNs alone from 36,277 with 56.1% coverage to 32,077 births with universal ITN coverage among primi,- and secundigravidae (Table 2.9).

Among first or second pregnancy, 25,243 of the 187,397 births or 13.5% ended in LBW births. Increasing the coverage of 2-dose IPTp-SP from the observed 30.4% to universal coverage (100%) has the potential of reducing LBW events among primi,- and secundigravida by an additional 21.6% or 5,459 LBW events from 25,243 to 19,784 births in Mali. The corresponding figures for universal coverage with ITNs were an additional 10.6% or 2,665 LBW events prevented from 25,243 to 22,578 births (Table 2.9).

Overall (in moderate and high malaria endemic areas, except Koulikoro), 213, 235 of the 394,843 births or 54.0% ended in anaemia (Table 2.8). This corresponding figure in first or second pregnancy was 63,951 of the 116,130 births or 55.1%. For universal coverage of IPTp, there is a potential of reducing anaemia among primi,-secundigravida by an additional 4.6% or 2,965 anaemia cases from 63,951 to 60,986 births. The corresponding figures for universal coverage with ITNs were 2.5% or 1,576 anaemia cases prevented from 63,951 to 62,375 (Table 2.9).

Table 2.8: Births with placental malaria , low birth weight or anaemia in all gravida

Transmission settings /Regions	Study sites	IPTp cover age, %	ITN cover age,%	Annual number of births*	Annual estimate of PM, No (%)**	Annual estimate of LBW, No (%) **	Annual estimate of anaemia, No (%)**
Low		**29.9**	**41.9**	**136,169**	**8,761 (6.4)**	**17,037 (12.5)**	**-**
Bamako	Banconi & Sabalibougou	42.0	59.8	79,401	1,191 (1.5)	10,265 (12.9)	-
Timbuktu	Timbuktu /Gao/Kidal	13.1	16.9	56,769	7,570 **(13.3)**	6,771 (11.9)	-
Moderate		**30.8**	**71.6**	**191,954**	**25,930 (13.5)**	**16,003 (8.3)**	**112,062 (58.4)**
Segou	San	28.8	80.0	102,537	10,338 (10.1)	7,641(7.5)	65,056 (63.4)
Mopti	Koro & Sangha‡	32.4	60.1	68,453	13,367 (19.5)	7,010 (10.2)	35,227 (51.5)
Mopti Djenne	Djenne	35.4	67.9	20,965	2,225(10.6)	1,352 (6.4)	11,779 (56.2)
High		**30.4**	**62.2**	**309,027**	**39,084 (12.6)**	**27,985 (9.1)**	**101,292 (32.8)**
Kayes	Kita	53.0	97.3	87,639	6,212 (7.1)	9,348 (10.7)	36,003 (41.1)
Koulikoro	Fana†	12.2	61.5	106,138	15,086 (14.2)	12,495 (11.8)	-
Sikasso	Bougouni	29.9	36.0	115,250	17,786 (15.4)	6,141(5.3)	65,290 (56.7)
Total		**30.4**	**60.7**	**637,150**	**73,775 (11.6)**	**61,025 (9.6)**	**213,355 (54.0)**

Notes: IPTp, intermittent preventive treatment; ITN, insecticide treated net; No, number ; PM, placental malaria; LBW, low birth weight;
* Annual estimate of life and stillbirths (see flow chart and method section).
**Point prevalence provided by the different surveys under the reported coverage of IPTp and ITNs.
† Haemoglonin level were not assessed during the Koulikoro survey
‡ Anaemia based on data from Koro only, no haemoglobin data from survey in Sangha.

Table 2.9: Births with placental malaria, low birth weight, and anaemia among first or second pregnancy

Transmission settings /Regions	Annual births*	IPTp cover age, %	ITN cover age, %	Annual estimate of PM, No (%)**	No of PM if 100 % IPTp	No of PM if 100 % ITN	Annual estimate of LBW, No (%) **	No of LBW if 100 % IPTp	No of LBW if 100 % ITN	Annual estimate of anaemia, No (%)**	No of anaemia if 100 IPTp	No of anaemia if 100 % ITN
Low	**40,050**	**27.1**	**42.2**	**1,922 (4.8)**	**1,034**	**1,612**	**4,396 (11.0)**	**3,374**	**3,731**			
Bamako	23,353	38.5	60.0	602 (2.6)	361	541	2,264 (9.7)	1,810	2,022			
Timbuktuctou/ Gao/Kidal	16,697	11.1	17.3	1,320 (7.9)	673	1,071	2,131 (12.8)	1,564	1,709			
Moderate	**56,457**	**30.3**	**62.6**	**12,340 (21.9)**	**7,030**	**11,127**	**7,010 (12.4)**	**5,453**	**6,298**	**28,952 (51.3)**	**27,443**	**28,227**
Segou	30,158	29.0	74.8	5,928 (19.7)	3,350	5,535	3,588 (11.9)	2,781	3,337	14,118 (46.8)	13,401	13,894
Mopti (Koro) †	20,133	30.9	47.2	5,510 (27.4)	3,151	4,796	3,043 (15.1)	2,373	2,628	10,611 (52.7)	10,015	10,232
Mopti (Djenne)	6,166	34.9	53.0	902 (14.6)	529	796	379 (6.1)	299	332	4,223 (68.5)	4,026	4,100
High	**90,890**	**31.8**	**58.2**	**21,985 (24.2)**	**12,446**	**19,338**	**13,838 (15.2)**	**10,958**	**12,549**	**34,999 (38.5)**	**33,543**	**34,148**
Kayes	25,776	55.3	96.6	3,217 (12.5)	2,167	3,186	5,220 (20.3)	4,414	5,167	15,316 (59.4)	14,818	15,283
Koulikoro‡	31,217	12.2	54.9	8,384 (26.9)	4,297	7,438	4,336 (13.9)	3,191	3,821			
Sikasso	33,897	32.1	32.1	10,384 (30.6)	5,982	8,714	4,283 (12.6)	3,561	3,561	19,683 (58.1)	18,725	18,865
Total	**187,397**	**30.4**	**56.1**	**36,248 (19.3)**	**20,510**	**32,077**	**25,243 (13.5)**	**19,784**	**22,578**	**63,951 (55.1)**	**60,986**	**62,375**

Notes: IPTp, intermittent preventive treatment in pregnancy ; ITN, insecticide treated net ; PM, placental malaria; LBW, low birth weight; No, number

* Annual estimate of life and stillbirths (see flow chart and method section).

Figures in parentheses for each region represent the observed prevalence from surveys. Figures in parentheses under the aggregated data for low, moderate, high and total are the annual number of LBW for that strata divided by the annual number of births at risk in that strata x 100.**

† Haemoglonin level were not assessed during the Koulikoro survey.

‡ Anaemia based on data from Koro only, Haemoglonin level were not assessed during the Sangha survey.

2.5 Discussion

Our results suggested that approximately 11.6% of the 637,150 births that occurred in Mali in 2009 may have had placental infection detectable by microscopy, and 9.6% resulted in LBW births, 25,243 of which were in primi and secundigravidae. Except in Bamako itself, the risk of placental malaria in 9 surveys conducted in other parts of Mali was considerable in women not receiving IPTp, ranging from 7.1% in Kayes, to 19.5% in Mopti (Koro & Sangha) (Figure 2.3). Placental malaria was associated with a higher risk of LBW (aPR, 1.46; 95% CI, 1.10-1.94), either due to preterm birth (aPR, 1.29; 95% CI, 0.81-2.05) or SGA (aPR, 1.25; 95% CI, 1.09-1.44) or the composite of the three outcomes (aPR 1.27; 95% CI, 1.12-1.45), or severe maternal anaemia (aPR, 1.91; 95% CI, 1.48-2.46) (Table 2.6). Interestingly, ITN ownership (93%) and use the night before the survey (65%) was among the highest reported for sub-Saharan Africa (van Eijk et al., 2013). This was consistent with the coverage reported in the recent demographic health survey conducted in 2012 (75.2%) (DHS, 2012-2013) as compared with that of 2006 with only 29% (DHS, 2006). However the coverage of 2-dose IPTp with SP was only 35%, well below the recent successive Roll Back Malaria (RBM) targets of 80% by 2010 and 100% by 2015, and even well below the Abudja target of 60% set in 2005 (van Eijk et al., 2013). The 2-dose IPTp regimen was strongly associated with lower risks of malaria and the composite of LBW or preterm birth when compared to women who did not receive any IPTp.

The relative low uptake of IPTp, suggests significant missed opportunities to further reduce the impact of malaria on newborn health. Our model suggested that increasing IPTp coverage to the audacious RBM target of universal coverage could result in a further 5,459 decrease in the number of LBW events among first and second pregnancies, a 22% boost in the overall reduction of LBW compared relative to that achieved at the current 35% level of coverage. The low uptake however is remarkable as it occurred despite significant financial and operational support from UNICEF and Save the Children and in areas where ITN distributions have been much more successful. A more recent study in Mali specifically designed to identify the main barriers to IPTp uptake showed a high percentage of women made at least two ANC visits (75%), with a significant proportion of women initiating ANC attendance in their first or second trimester (88%), but poor adherence to policy guidelines

of antenatal staff resulting in many missed opportunities to deliver SP during scheduled antennal visits in the 2nd and 3rd trimester (Hill, 2013). Furthermore, it is also showed that only 0% to 24.5% of women receiving SP received it as directly observed therapy. Both ANC attendance patterns among women and factors affecting the delivery of care at ANC contribute to the low effectiveness reported and pointing to the need to improve the quality of IPTp provision (Webster et al., 2013a) .

Interestingly, a similar high prevalence of placental infection (13.3 %) among women not protected by IPTp was observed in Timbuktu in the north of Mali. The high risk of placental malaria was unexpected because the survey had missed the rainy season and was conducted during a 3 month period towards the end of the dry season starting 5 months after the rains had ceased (Figure 2.6). Furthermore, this is a semi-arid region with an annual rainfall averaging ~300 mm per year that until recently had predominately low or even epidemic malaria transmission (Doumbo O., 1991). The most likely explanation of this high prevalence in the dry season is the recent shift in ecological conditions in northern Mali resulting from the introduction of large scale irrigation projects, which have turned parts of the Sahara desert into paddy fields for rice farming, sustaining malaria transmission throughout the year (Koita et al., 2012). In addition, parasites acquired during the rainy season may sequester in the placenta and persist for several months into the dry season, especially among women not using IPTp-SP. This is also suggested by the observation from some of the other surveys included in this study, such as in Mopti, Sangha (2006-2007) that started during the transmission season but continued in the dry season. These finding are also consistent with observational cohort studies using weekly follow-up in areas with low transmission from the Thai-Burmese border (McGready et al., 2004).

Ecological analysis showed similar prevalence of LBW across transmission settings (Figure 2.3) and no clear association was found between the risk of LBW among primi,- and secundigravida or all gravida and transmission intensity, as measured by the prevalence of maternal peripheral malaria among women who did not receive IPTp-SP using meta-analysis approach (Figure 2.4 & Figure 2.5). The prevalence of malaria at delivery in all other surveys, including in Timbuktu, exceeded 10%, thus the variation in malaria transmission intensity may have been too small to allow for a meaningful analysis of the trend between LBW and

malaria transmission intensity. Furthermore, LBW is multifactorial (Guyatt and Snow, 2004) and other determinants not assessed will have contributed to LBW.

By contrast to previous studies in Timbuktu or other parts in the North of Mali (Doumbo O., 1991, Bernabeu et al., 2012, Koita et al., 2012), we did not find *P. vivax* malaria. This does not exclude the presence of *P. vivax*, and malaria control strategies should probably take the presence of *P. vivax* into account in this region.

The study has several limitations that need to be considered. First, although the surveys where designed to occur during the peak transmission season some were conducted toward the tail end of the transmission season or even in dry season, such as in Timbuktu and Koulikoro. Ideally, surveys planned to capture the burden of malaria in pregnancy should be designed to capture data throughout the year, especially in areas with highly seasonal transmission such as in Mali where the start and duration of the seasons differ by geographical region and from year to year. Second, the surveys were conducted across a period of five years and the earlier surveys conducted over 8 years ago may no longer reflect the current coverage or the prevailing disease burden as recent reports suggest markedly decrease in malaria prevalence in sub-Saharan Africa (O'Meara et al., 2010, Pond, 2013). Third, even though all regions were represented and the 9 sites represented the wide range of transmission settings in Mali, the study was not designed as a national random cluster survey and sites were chosen based on ongoing program activities by the Malaria Research and Training Center and may thus not be representative of Mali as a whole and overestimate coverage of ITNs and IPTp. Furthermore, some of the traditional agricultural irrigation areas were not included, such as Niono, Markala, Macina, Selingue, where malaria transmission is bimodal. Fourth, the study was clinic based and thus only included women who had access delivery units, where 45% of births in Mali occur at home in the community (DHS, 2012-2013). Lastly, data captured at delivery does not exclude earlier infections during pregnancy that may have been cleared. We also used microscopy for the diagnosis of malaria, which is known to be less sensitive to capture infection than PCR and placental histology (Fried et al., 2012, Singer et al., 2004, Taylor et al., 2010). Thus, the true prevalence of malaria detectable at delivery and impact on birth outcome may be considerably higher.

2.6 Conclusion

Despite these limitations, this is the first comprehensive study of the burden of PAM throughout Mali and the results suggest that malaria in pregnancy is a major problem affecting over 600,000 births annually. Furthermore large scale rice irrigation projects in the semi-arid regions in northern Mali places women at risk throughout the year. Prospective burden surveys should thus aim to capture data throughout the year, ideally using sensitive diagnostic tests to detect malaria infection. The high ownership of ITNs is an important achievement; however, major further improvements can be achieved by scaling up the use of IPTp with SP to reduce the risk of severe maternal anaemia and birthweight.

Chapter 3: Superiority of three over two doses of intermittent preventive treatment with sulfadoxine-pyrimethamine for the prevention of malaria during pregnancy in Mali: a randomized controlled trial

3 Superiority of three over two doses of intermittent preventive treatment with sulfadoxine-pyrimethamine for the prevention of malaria during pregnancy in Mali: a randomized controlled trial

3.1 Introduction and background

Intermittent preventive therapy in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) is effective in reducing the risk of placental malaria, low birth weight (LBW) and severe maternal anaemia (Ter Kuile et al., 2007, WHO/AFRO, 2004) and together with insecticide treated nets, the main strategy for the control of malaria in pregnancy in Africa (WHO/AFRO, 2004). IPTp consists of the administration of full curative doses of an efficacious antimalarial drug given presumptively in the 2nd and 3rd trimester at least one month apart. It provides intermittent clearance or suppression of existing asymptomatic infections from the placenta ('treatment effect') and 'post-treatment' prophylaxis by preventing new infections through the maintenance of suppressive drug-level for up to 6 weeks in areas with low parasite resistance (White, 2005, WHO/AFRO, 2004).

Presently, SP is the only antimalarial recommended for IPTp (WHO, 2013). At the time of study, the World Health Organization (WHO) recommended at least 2 curative doses of SP in HIV negative women, and 3 doses for HIV-positive women, not protected by cotrimoxazole (Ter Kuile et al., 2007, WHO/AFRO, 2004, World Health Organization, 2004, World Health Organization, 2006). Although SP resistance has increased to high levels in some areas of southern and eastern Africa, resistance in most of western Africa is still low. Furthermore SP has now been reserved for use as IPT and the reduced drug pressure may prolong the longevity of this very valuable drug in areas with low to moderate resistance (Ter Kuile et al., 2007, World Health Organization, 2006). IPTp with SP is thus likely to remain the main stay of malaria control in pregnancy for several years in these regions.

In Mali, the two-dose strategy was adopted by the National Malaria Control Program (NMCP) in 2003. Mali has highly seasonal malaria transmission, moderate levels of ITNs use and low levels of SP resistance (Diallo et al., 2006, Thera et al., 2005). Recent survey from 2005-2007 involving 1,696 pregnant women of all parities showed that placental infection was very common among women who had received the full two-dose regimen of IPTp (23%; Kayentao et al., unpublished data), consistent with findings from an earlier trial showing that

2 dose IPTp-SP, although much more effective than chloroquine prophylaxis, was associated with high risk of placental infections during the transmission season, especially in women who completed the second dose of IPTp-SP early in the third trimester (Kayentao et al., 2005). This suggests that two doses may provide insufficient protection against reinfections later in the third trimester, a period of rapid in-utero growth.

Three trials have shown that additional doses of SP add significant benefit over the two dose regimen among HIV-infected primi,- and secundigravidae (Ter Kuile et al., 2007). In HIV-negative women, the beneficial effect of 3 or more doses is less clear. We conducted an open-label, individually randomized controlled trial comparing the efficacy and safety of the 2-dose regimen of IPTp-SP with three doses of SP in the prevention of placental malaria, maternal anaemia and low birth weight in Mali.

3.2 Methods

This was an open-label, individually randomized, controlled trial comparing the efficacy and safety of the 2-dose regimen of IPTp-SP with three doses of SP in the prevention of placental malaria, maternal anaemia and low birth weight in Mali. The study was conducted from April 2006 to March 2008 in two health facilities in Bla District, located 320 kilometers east of Bamako in Ségou Region, Mali, an area with highly seasonal malaria transmission. The two health facilities serve a population of approximately 39,000. Women of all gravidity scheduled to receive their first dose of IPTp-SP were eligible if they were aged 14-45 years, were between 16-26 weeks gestation (by fundal height) and had no history of antimalarial or cotrimoxazole use in the previous month. Women with a serious illness requiring admission, with severe anaemia (Hb<7 g/dL), with known HIV infection, or who were planning to deliver elsewhere were excluded.

3.2.1 Randomization, masking and treatment allocation

Women were randomly assigned to one of the two study groups by the study clinicians. Allocation concealment was achieved by keeping 20 allocation slips with pre-assigned study allocations (10 per arm) into opaque containers. Sequential participants were asked to draw one allocation slip from the container without possibility of replacement. The randomization sequence was stratified by clinic and used permuted balanced block randomization (block-size of 20). Study drugs were provided by the study clinicians. All nurses, midwifes in the

delivery units, and all laboratory staff were unaware of the treatment allocation during the trial.

3.2.2 Endpoints

The primary endpoint was placental malaria infection (asexual stage parasites, any species). Secondary efficacy endpoints included maternal malaria determined by peripheral smear; mean haemoglobin, maternal anaemia (Hb <11 g/dL) and moderate-to-severe anaemia (Hb<8.0 g/dL); mean birthweight and low birth weight (<2500 g); mean gestational age and preterm delivery (gestational age <37 weeks); and low placental weight (<500 g). Safety endpoints included severe maternal skin reactions (e.g. Stevens-Johnson syndrome and toxic epidermal necrolysis), congenital malformations assessed at birth and 30 days, and neonatal icterus and vomiting by day 7 and day 30 of life.

3.2.3 Procedures

A questionnaire was administered at enrolment to collect information on socio-demographic characteristics, maternal height, bed net use, obstetric and clinical histories, and drug use during the pregnancy. A blood sample was obtained by fingerpick for malaria smears, and haemoglobin concentrations (Hemocue® 201; Anglholm, Sweden).

Women were asked to return to the clinic for monthly follow-up or in-between for any unscheduled sick-visits. If women did not return for their scheduled visit they were visited at home. During each visit, women were asked about signs and symptoms and examined for skin rashes. If a woman had documented fever (T>=37.5 ^{o}C), the axillary temperature was measured and a malaria blood smear taken.

Within 2 hours after delivery a finger prick sample was taken for haemoglobin concentration and malaria parasitemia. Placental blood was collected by incision from the maternal side of the placenta for malaria blood smears. The newborns were examined by the study physicians for congenital malformations, and the gestational age was assessed using the Ballard score. Infants were weighed using a digital scale. Mothers and neonates were visited at home by the study staff a week and a month after delivery to examine the newborn. The study was monitored monthly by independent internal clinical monitors on behalf of the Malaria Research and Training Centre in Bamako.

3.2.4 Interventions

Each treatment dose consisted of 1,500 mg of sulfadoxine and 75 mg of pyrimethamine (total of 3 tablets). Women in the 3-dose group received the first dose between 16-24 weeks gestation, the second dose between 20-32 weeks, and the third dose no later than the 36 weeks gestation. Women in the 2-dose group also received the first dose between 16 and 24 weeks and the second dose between 25 to 36 weeks. Doses were administered at least one month apart. All drugs were administered directly by the study physicians and women were observed for 30 minutes following dosing. If women vomited within 30 minutes the full treatment dose was repeated. Study participants were asked to avoid self-medication of antimalarials other than the study medication until completion of follow-up at 6 weeks post-partum. All women received ferrous sulphate (200 mg containing 60 mg of iron) and folic acid (0.4 mg) daily starting two weeks after each SP dosing as recommended by the Malian Ministry of Health. If malaria occurred during follow-up visits, oral quinine was given 600 mg three times daily over 7 days.

3.2.5 Laboratory assessment

Thick blood smears were stained with 4% Giemsa for 20 minutes. Parasite densities were determined by counting the number of asexual parasites per 300 WBCs assuming a WBC count of 7,500/uL. A smear was determined to be negative if no parasites were identified after review of 100 high-power fields. Slides were read by an experienced microscopist blinded to the treatment allocation. Quality control was done on ten percent of slides which were read by an expert reader blinded to initial results and to treatment allocation.

3.2.6 Sample size and data analysis

The study was designed to detect a 7.5% reduction in the prevalence of placental malaria from 15% in the 2-dose group. A sample size of 304 women in each treatment arm was required (alpha 0.05, power 80%); this was increased to 406 to account for 5% non-compliance and 20% loss-to-follow-up.

Data were double entered and validated using Epi-Info (version 6.04b; CDC) and analyses conducted using Stata (version 12) and SPSS (version 17). The efficacy analysis was on an intention-to-treat basis. The safety analysis for adverse skin reactions was performed on a modified intention-to-treat basis and included only women who took at least one dose of

study drug. The analysis of safety endpoints measured at or after delivery was done on a per-protocol basis. The impact of the treatment on dichotomous endpoints at delivery was compared using robust stepwise backwards elimination Poisson regression to obtain adjusted prevalence ratios (APR). We also conducted exploratory analyses of potential effect modification by season, by gravidity and by ITN use.

3.3 Ethical approval

The study was approved by the ethical committee of the Faculty of Medicine, Pharmacy and Dentistry of the University of Bamako. Consent procedures were in the local language (Bambara). All women participating in the study gave written informed consent after being informed about the study and procedures.

3.4 Results

3.4.1 Study population characteristics at enrollment

A total of 814 pregnant women were enrolled from 21 April 2006 to 19 February 2008; 413 in the 3-dose arm and 401 in the 2-dose arm (Figure 3.1). The baseline characteristics were similar across the two treatment arms (Table 3.1). Thirty-one of the 814 women (3.8%) were lost before delivery because of travel (6 [0.7%]), consent withdrawal (14[1.7%]), and non-compliance with the follow-up schedule (11 [1.4%]). The percentage lost-to-follow-up was 2.6% (11) in the 3-dose arm and 4.9% (20) in the 2-dose arm (P=0.09). The remaining 783 (96.2%) women were followed successfully until delivery and contributed to the intention-to-treat analysis; 16 (4.0%) of the 400 women in the 3-dose group did not receive their third dose and 3 (0.8%) of 383 in the 2-dose group received only one dose and were excluded from the per-protocol analysis (Figure 3.1).

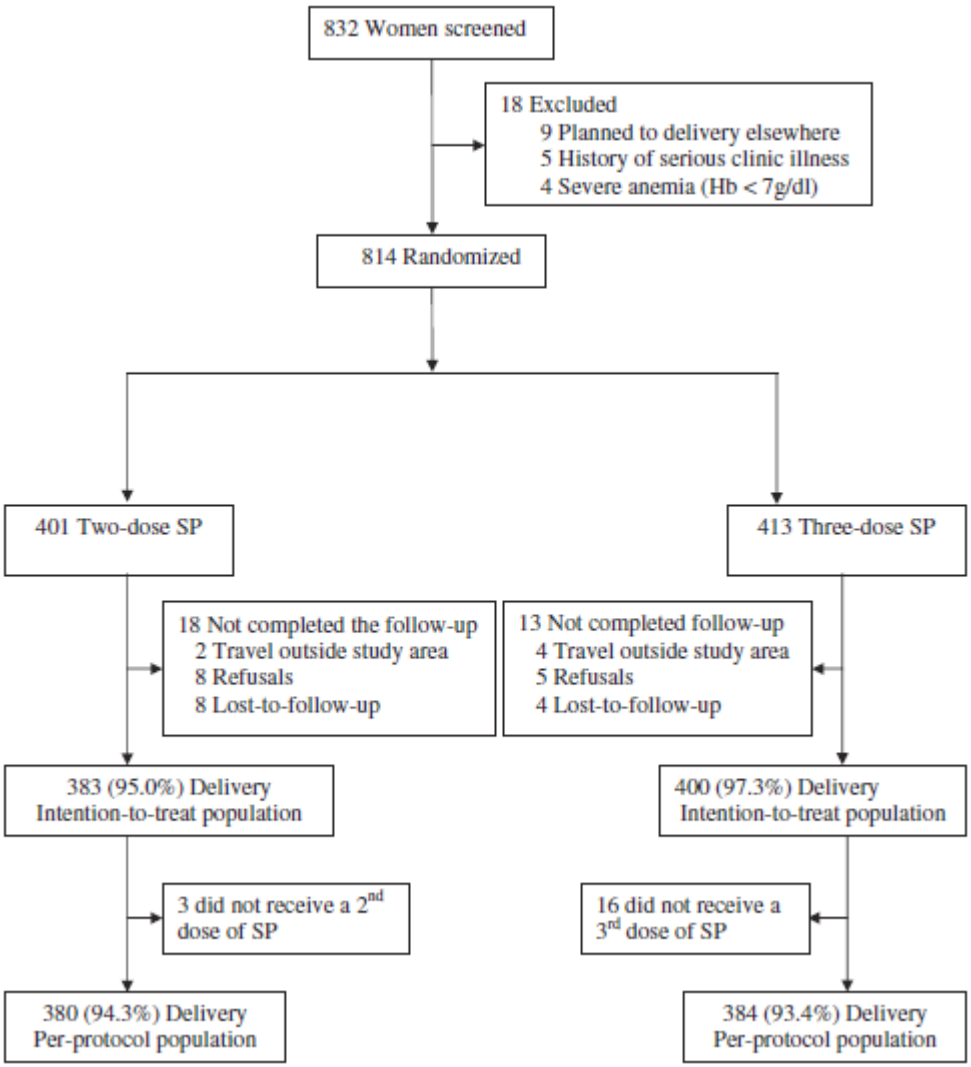


Figure 3.1: Trial Profile

***Figure note**:* Thirty-one of the 814 enrolled women (3.8%) were lost before delivery because of travel (6 [0.7%]), consent withdrawal (13[1.6%]), or non-compliance with the follow-up schedule (12 [1.5%]). The percentage lost-to-follow-up was 3.1% (13) in the 3-dose arm and 4.5% (18) in the 2-dose arm (P=0.31). The remaining 783 (96.2%) women were followed successfully until delivery and contributed to the intention-to-treat analysis; 16 (4.0%) of the 400 women in the 3-dose group did not receive their third dose and 3 (0.8%) of 383 in the 2-dose group received only one dose and were excluded from the per-protocol analysis.

Table 3.1 : Baseline characteristics on enrollment by IPTp group

Characteristics	2-dose SP (n=401)	3-dose SP (n=413)	All women (n=814)
Age in years			
mean (SD)	24.5 (6.1)	24.5 (6.0)	24.5 (6.1)
Range	14-43	15-45	14-45
Age<20 years, %	26.2	22.4	24.3
Height <150 cm, %	2.0	1.5	1.7
Weight <50 Kgs, %	7.9	6.3	7.1
Gestational age in weeks			
mean (SD)	21.6 (2.6)	20.8 (2.6)	21.18 (2.6)
Range	16-26	16-26	16-26
Married, %	95.3	95.4	95.3
Gravidity			
Median, range	3 (1-14)	3(1-13)	3(1-14)
Primi and secundi-gravidae, No. (%)	42.1	41.5	41.8
Attended ANC this pregnancy prior to enrollment, No. (%)	128 (32.0)	129 (31.2)	257 (31.7)
Axillary temperature ≥37.5^{o}, %	27.7	22.0	24.8
Sleep under bed net, No. (%)	226 (56.4)	225 (54.5)	451(55.4)
Sleep under ITN, No. (%)	72 (18.0)	88 (21.3)	160 (19.8)
Sleep under ITN last night, No. (%)	59 (14.7)	77 (18.4)	136 (16.7)
Haemoglobin in g/dL			
mean (SD)	10.1 (1.5)	10.2 (1.5)	10.2 (1.5)
Moderate anaemia (<11 g/dL), %	72.3	67.3	69.78
Severe anaemia (<8 g/dL), %	4.2	2.2	3.2
Positive peripheral malaria smear, %	25.7	27.6	26.7
Clinical malaria, %	12.0	9.2	10.6
Parasite density /uL, geometric mean (95 CI) [a]	1,128 (765, 1663)	1843 (1351, 2514)	1,464 (1145, 1872)

Notes: Data are no. (%) or enrolled women, unless otherwise indicated. SP, Sulfadoxine-pyrimethamine; SD, Standard deviation; ANC, Antenatal Clinic; CI, Confidence Interval.
Clinical malaria defined as positive peripheral smear in the presence of documented fever (≥37.5 ^{o}C) or history of fever within the last 24 hours.
[a]Among malaria positive women only

3.4.2 Efficacy of regimens

Overall, 4.8% (20 of 413) and 7.2% (29 of 401) of women in the 3-dose and 2-dose group, respectively, had at least 1 episode of clinical malaria (confirmed by microscopic examination) and were treated with quinine (APR, 0.79; 95% confidence interval [CI], 0.56–1.12). At delivery, the prevalence of placental malaria in the 3-dose group was half that in the 2-dose group (8.0% vs 16.7%; APR, 0.48; 95% CI, 0.32–0.71) (Table 3.2). Interaction models indicated that the beneficial effect was evident in all gravidae groups and in ITN users and nonusers (Table 3.2). In the 2-dose group, women enrolled before 24 weeks gestation were more likely to have placental malaria than were those enrolled later (APR, 1.21; 95% CI, 1.06–1.37). This was not apparent in the 3-dose group (APR, 1.07; 95% CI, 0.88–1.32). The risks of LBW and preterm birth were also halved, but the prevalence of moderate and severe anaemia was similar in the 2 treatment arms (Figure 3.2 & Table 3.2). The impact on LBW was similar across gravidae groups and ITN users and nonusers, but the impact on mean birth weight was greater in primi- and secundigravidae (who experienced an increase in mean birth weight of 99 g) than in multigravidae (who experienced an increase of 50 g) and was greater in non-ITN users (who experienced an increase of 97 g) than in ITN users (who experienced a decrease of 27 g) (Table 3.2). The observed differences between the 2 treatment arms were greatest during and shortly after the malaria season (Figure 3.3). There were no differences between treatment groups with respect to foetal loss (Table 3.2). The frequency of single-nucleotide polymorphisms (SNPs) was determined in a small number of positive samples at delivery. The frequency of the *dhfr/dhps* quadruple mutant genotype was 85.7% (6 of 7 samples) and 55.6% (5 of 9 samples) in the 2-dose and 3-dose arms, respectively, at delivery (P =0 .31), and no quintuple mutation was observed.

Table 3.2: Primary and secondary outcomes at delivery IPTp group

		Number of subject (%)					
Characteristics		**2-dose SP**	**3-dose SP**	**Unadjusted absolute risk difference, % (95% CI)**	**Unadjusted difference in mean or Prevalence Ratio (95% CI)**	**Adjusted difference in mean or Prevalence Ratio (95% CI)**	**[a]P-value**
Primary outcome							
Placental blood smear No. positive (%)	All gravidae	64/383 (16.7)	32/398 (8.0)	8.7 (4.2, 13.3)	0.48 (0.32, 0.72)	0.48 [b] (0.32, 0.71)	<0.001
By gravidae group	Primi-secundi	32/159 (20.1)	16/165 (9.7)	10.4 (2.7, 18.1)	0.48 (0.28, 0.84)	0.47 [b, c] (0.27, 0.82)	0.007
	Multigravidae	32/284 (14.3)	16/233 (6.8)	7.4 (1.8, 13.0)	0.48 (0.27,0.85)	0.48 [b, c] (0.27, 0.85)	0.012
By ITN use	ITN-user	14/72 (19.4)	5/85 (5.9)	13.6 (3.1, 24.0)	0.30 (0.11, 0.80)	0.30 [b, d] (0.12, 0.80)	0.016
	Non ITN-user	50/311 (16.1)	27/313 (8.6)	7.5 (2.3, 12.6)	0.53 (0.36,0.83)	0.53 [b, d] (0.34, 0.82)	0.005
Secondary outcome							
Positive peripheral smear No. positive (%)	All gravidae	77/383 (20.1)	43/400 (10.8)	9.4 (4.3, 14.4)	0.53 (0.37, 0.75)	0.54 [b] (0.38, 0.77)	0.001
Haemoglobin concentration	All gravidae						
Mean (SD), g /dL		10.9 (1.5)	11.1 (1.5)		0.14 (-0.08, 0.35)	0.02 [e] (-.01, 0.1)	0.260
Hb<11 g/dL, No. (%)	All gravidae	188/383 (49.1)	172/400 (43.0)	6.1 (-0.8, 13.1)	0.88 (0.75, 1.01)	0.89 [e](0.76, 1.04)	0.144
Hb<8 g/dL, No. (%)	All gravidae	8/383 (2.1)	13/400 (3.3)	-1.2 (-7.5, 1.6 %)	1.7 (0.7, 4.1)	1.8 [e](0.8, 4.1)	0.172
Birth weight[f]							
LBW, No. (%)	All gravidae	48/360 (13.3)	25/378 (6.6)	6.7 (2.4, 11.0)	0.49 (0.31, 0.78)	0.50 [f] (0.32, 0.79	0.003
By gravidae group	Primi-secundi	32/151 (21.2)	15/151 (9.9)	11.3 (3.2, 19.3)	0.47 (0.24, 0.83)	0.46 [c, f] (0.26, 0.82)	0.008
	Multigravidae	16/209 (7.7)	10/227 (4.4)	3.3 (-1.2, 7.7)	0.57 (0.26, 1.24)	0.57 [c, f] (0.27, 1.23)	0.152

By ITN use	ITN-user	8/68 (11.8)	5/80 (6.3)	5.5 (-3.8, 14.8)	0.53 (0.18, 1.55)	0.53 [d, f] (0.19,1.46)	0.219
	Non ITN-user	40/292 (13.7)	20/298 (6.7)	6.9 (2.1, 11.8)	0.48 (0.29, 0.82)	0.50 [d,f] (0.30, 0.82)	0.006
Mean (SD), grams		2892 (464)	2964(428)		72.3 (7.8, 136.8)	70.37[f](8.53, 132.2)	0.026
By gravidae group	Primi-secundi	2762 (427)	2853 (457)		91.1 (-9.2, 191.3)	98.50 [c, f](1.15, 195.9)	0.047
	Multigravidae	2986 (468)	3038 (292)		52.4 (-28.7, 133.5)	49.9 [c, f] (-30.8, 130.6)	0.219
By ITN use	ITN-user	2937 (515)	2911 (331)		-26.1 (-112.7, 164.9)	-27.9 [d,f] (-164.3, 108.5	0.686
	Non ITN-user	2882 (452)	2979 (450)		97.1 (24.1, 170.1)	89.6 [d,f] (19.2, 159.9)	0.013
Gestational age	All gravidae						
Preterm, No. (%)		32/359 (8.9)	12/376 (3.2)	5.5 (2.3, 9.2)	0.35 (0.18, 0.68)	0.37 [f] (0.19, 0.71)	0.003
Mean (SD), weeks	All gravidae	38.6 (1.6)	38.8 (1.5)		0.2 (-0.03, 0.4)	0.1 [f] (-0.03, 0.4)	0.101
Placental weight[f]							
Placental weight <500 g, No. (%)	All gravidae	113/359(31.5)	90/375 (24.0)	7.5 (1.01, 13.4)	0.76 (0.60, 0.97)	0.76 [f] (0.60, 0.97)	0.024
Mean (SD), grams	All gravidae	530 (114)	535 (316)		-4.5 (-28.6, 37.6)	4.86 [f] (-38.0, 28.3)	0.774
Pregnancy loss[g], No. (%)	All gravidae	16/383 (4.2)	20/400 (5.0)	-0.8 (-3.7, 2.1)	1.2 (0.6, 2.3)	1.12 [g] (0.59, 2.12)	0.723
Perinatal deaths[h], No. (%)	All gravidae	8/383 (2.71)	10/400 (2.5)	-0.4 (-2.5, 1.7)	0.84 (0.20, 2.40)	0.78 [h] (0.12, 2.16)	0.342
Neonatal deaths, No. (%)	All gravidae	6/360 (1.7)	6/376 (1.6)	0.1 (-1.7, 1.9)	0.95 (0.31, 2.9)	0.88 [h] (0.29, 2.63)	0.817

Notes: ANC, antenatal clinic; CI, confidence interval; ITN, insecticide treated net; LBW, low birth weight; SD, standard deviation.

[a] Bivariate endpoints: Poisson regression with robust variance, continuous endpoints: multiple linear regression

[b] djusted for gravidity (where applicable), season of delivery, age, malaria at enrolment

[c] P value for difference of treatment effect by gravidity strata (interaction term): placental malaria, 0.96; LBW, 0.68; mean birth weight, 0.52.

[d] P value for difference of treatment effect by ITN use (interaction term): placental malaria, 0.32; LBW, 0.79; mean birth weight, 0.15.

[e]Adjusted for gravidity, season of delivery and haemoglobin at enrolment

[f] adjusted for gravidity, season of delivery and maternal weight at enrolment

[g] Spontaneous abortion or stillbirth (adjusted for gravidity and season)

[h] Pregnancy loss or early neonatal death in the first week of life (adjusted for gravidity and season)

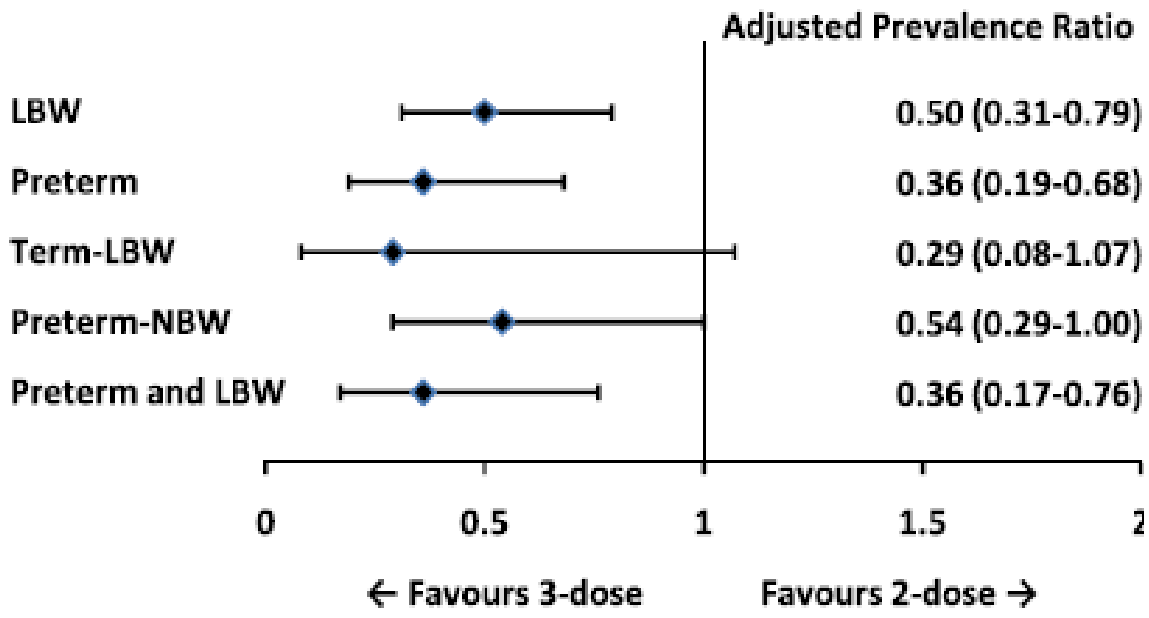


Figure 3.2 : Treatment effect on low birth weight and preterm delivery.
Note: LBW, low birth weight (<2500 g); NBW, normal birth weight (>=2500 g); preterm delivery, <37 weeks gestation; term delivery, >=37 weeks gestation.

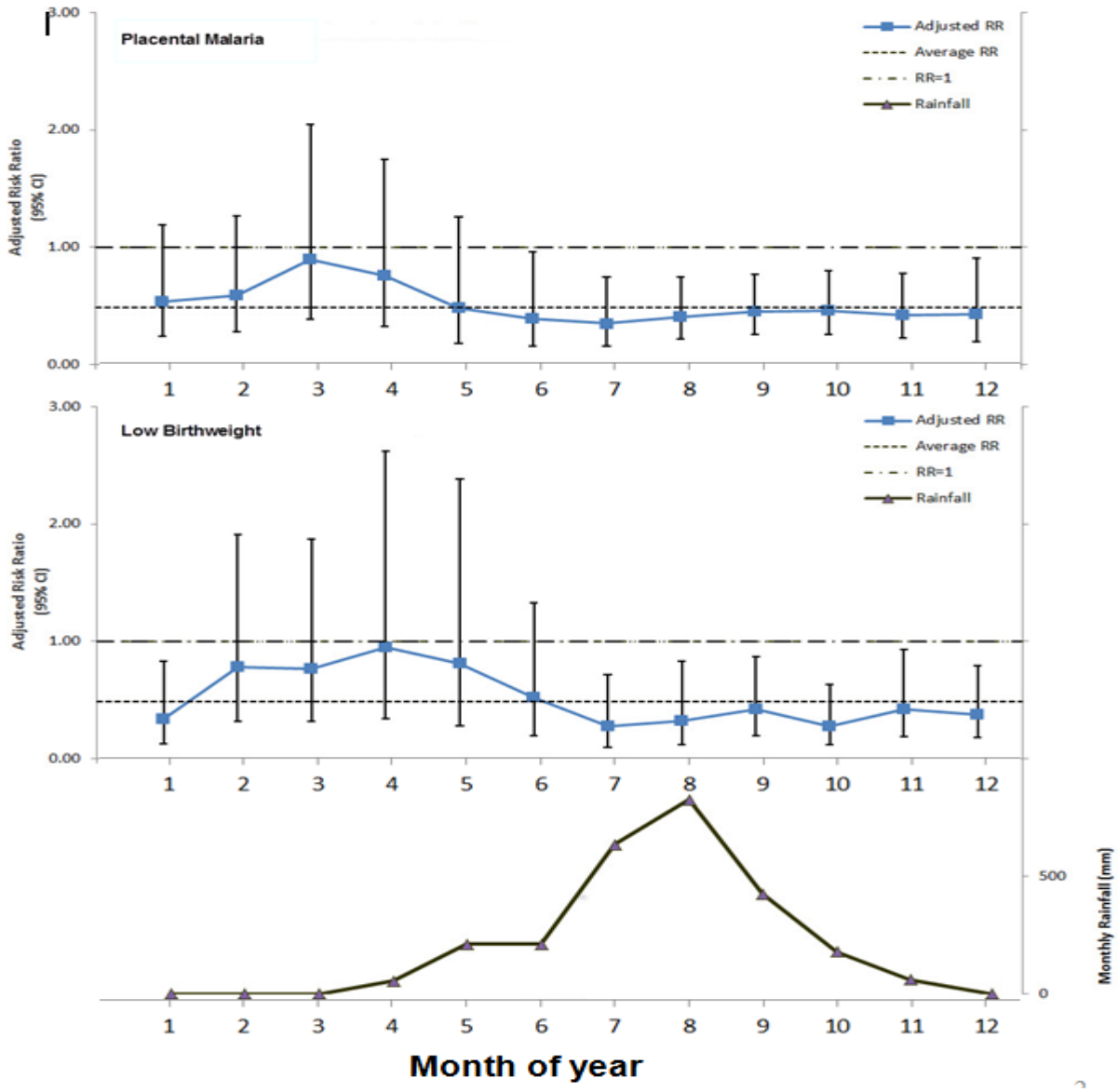


Figure 3.3: The treatment effect of 3,-versus 2-dose IPTp on placental malaria (upper graph) and LBW (lower graph) by malaria transmission season

***Figure note*:** Three-month moving averages of the prevalence ratios (PR) obtained from Poisson regression models adjusted for gravidity and baseline parasitemia (placental malaria) and maternal weight (LBW). The error bars represent the 95 confidence intervals. The top horizontal line (—¨—) depicts a prevalence ratio of 1 indicating 'no difference' between treatment groups. Point estimates below this line indicate a beneficial effect in favor of the 3-dose group. The effect is statistically significant if the upper error bar does not cross the PR value of 1. The lower horizontal line (- - -) at PR values of 0.48 and 0.50 indicates the average adjusted prevalence ratio observed over the duration of the study. Data reflects the average estimates per month of the 2 year period of the study. Malaria transmission is depicted by the average monthly rainfall in the two study locations during the study period. Significant beneficial treatment effect on placental malaria was observed during the 7 month period starting in June, i.e. one month after the first rainfall and ending one month into the dry season (December) (APR 0.43, 95% CI 0.27-0.69, P=0.001). A smaller non-significant reduction was observed in the dry season from January to May (APR 0.68, 95% CI 0.33-1.40, P=0.29) (P-value interaction term 0.33). The corresponding values for LBW were: APR 0.37, 95% CI 0.20-0.68, P = 0.001 and APR 0.81, 95% CI 0.39-1.68, P=0.58) (P-value interaction term 0.035).

3.4.3 Adverse events in mothers, neonates and infants

No severe skin rashes were observed during the study. One mother died from post-partum hemorrhage in the two-dose group. There were 4 (0.6%) congenital abnormalities detected (3 poly-dactily and 1 club-foot) at the time of birth: 3 in the 2-dose group and 1 in the 3-dose group. There were no differences in the prevalence of jaundice, history of fever or history of vomiting in the neonates at day 7 or day 30 (Table 3. 3).

Table 3.3: Adverse events among newborns by study group

	Proportion (%) of newborns				
	2 -dose SP	3-dose SP	Absolute risk difference (95% CI)	Prevalence Ratio (95%CI)	
Adverse events	n/N (%)	n/N (%)			P-value
Icterus					
Day 7,	9/365 (2.5)	10/375 (2.7)	-0.2%(-2.4, 2.1)	1.1(0.4, 2.6)	0.9
Day 30	0	0			
Congenital birth abnormalities	3/359) [a]	1/362 [b]			
Fever Day7	10/365 (2.7)	17/375 (4.5)	-1.8%(-4.4, 0.8)	1.6(0.76, 3.6)	0.19
Day 30	9/361(2.5)	11/375(2.9)	-0.4%(-2.7, 1.9)	1.2(0.49, 2.8)	0.71
Vomiting Day 7	7/365 (1.9)	7/375 (1.9)	0%(-1.2, 2)	1.0 (0.36,	0.95
Day30	5/361 (1.4)	9/375 (2.4)	-1%(-2.9, 0.9)	2.9)	0.32
				1.7(0.6, 5.1)	

[a] Poly-dactily; [b]club-foot

Notes: CI, confidence interval; data are percentage, unless otherwise indicated.
P-values presented here compared percentage of adverse events between treatment arms on day 0 and day 30 separately.

3.5 Discussion and conclusion

Three doses of IPTp with SP was considerably more effective in reducing maternal and placental malaria, low birth weight and premature delivery than the 2-dose regimen in this area with low SP resistance and highly seasonal malaria. The extra dose of SP was well tolerated. Size at birth and prematurity are important risk factors for infant morbidity and mortality, and both are associated with permanent deficits in childhood growth and neuro-cognitive development and performance in later life (Medicine/National and Sciences, 1990, WHO, 1995, Teberg et al., 1988, McCormick, 1985, Williams et al., 1982). Our findings thus have important public health implications.

The reduction in placental malaria is not surprising, because the 3-dose group received their last dose, on average, 1 month closer to term, clearing existing infections and reducing the susceptibility to new infections at term by providing an extra period of post-treatment prophylaxis of 4–6 weeks. The latter half of the third trimester is a period of considerable foetal growth, when at least 25% of the total foetal weight gain occurs in healthy pregnancies.

This explains the large impact of the third dose on birth weight, despite the fact that the control group had received the 2-dose regimen, which itself was highly effective in a previous trial in Mali (Kayentao et al., 2005). The observed difference in mean birth weight of 66 g is comparable to that reported in meta-analyses of previous trials of ITNs (55 g) and of 2-dose IPTp (79 g) (Gamble et al., 2007, Ter Kuile et al., 2007). Our data add to the growing body of evidence showing that more than 2 doses of SP are required to provide adequate protection to pregnant women through the third trimester. This is the first trial that compares the 2-dose regimen with more frequent dosing schedules in western Africa. However, in eastern and southern Africa, there have been 4 previous trials comparing 2-dose IPTp-SP with more-frequent dosing, conducted over a 15-year period in areas representing a wide range of SP resistance; 4 of these involved HIV-infected women (who were not taking cotrimoxazole) (Filler et al., 2006, Hamer et al., 2007, Luntamo et al., 2010, Parise et al., 1998) and 3 of which involved HIV-negative women (Filler et al., 2006, Luntamo et al., 2010, Parise et al., 1998), 2 of which studies restricted recruitment to primi- and secundigravidae only (Filler et al., 2006, Parise et al., 1998). We pooled the results from our study with those of the 2 other trials involving HIV negative women that reported results for primi- and secundigravidae separate from the

multigravidae in a meta-analysis. These data show remarkably consistent findings across the studies, with no evidence of heterogeneity (I^2=50% for the effect on mean birth weight and 20% for LBW) (Kayentao et al., 2011). The other 3 trials all showed that a positive impact on mean birth weight was associated with more-frequent dosing, with increases in mean birth weight ranging from 57 g to 80 g, compared with 91 g among primi and secundigravidae in our current study (summary estimate across the 3 trials, 80 g; 95% 15–145 g; P = 0.02). The summary estimate for LBW gives an APR (95% CI) of 0.64 (0.43–0.95) (P =0.03), including the 0.47 estimate among the primi- and secundigravidae in our current trial. Thus, it is unlikely that the large beneficial impact observed in our study is a chance finding. The evidence from the other trials conducted in eastern and southern Africa (Filler et al., 2006, Luntamo et al., 2010, Parise et al., 1998) suggests that the benefits of more-frequent dosing may also apply in areas with higher, albeit moderate, levels of SP resistance. With increasing drug resistance, the minimum inhibitory concentration at which parasite growth is inhibited increases, and the time window for effective drug concentrations that fall below these levels decreases. This results in a progressive shortening of the duration of the suppressive prophylactic effect after treatment (White, 2005). Parasites with triple DHFR mutations have a 1000-fold reduction in susceptibility to pyrimethamine, which translates into a reduction in the duration of post-treatment prophylaxis of 1 month, compromising the efficacy of the 2-dose regimen and requiring more-frequent dosing (Ter Kuile et al., 2007, White, 2005). This study was conducted in an area with low SP resistance and in the same region as our ongoing studies of malaria in pregnancy. In this area, the prevalence of *dhfr/dhps* quadruple mutant genotype among asymptomatic parasitemic pregnant women attending the clinic for antenatal care was only 28% prior to 2009. At this level of molecular resistance, the *in vivo* response to SP is excellent; weekly follow-up showed that only 3.2% of 268 women were parasitemic again by day 42 (Coulibaly et al., 2014) . It is likely that repeated dosing with SP as part of IPTp in women may select for a subpopulation of clones and strains with a less sensitive phenotype. Although the number of successfully genotyped samples from parasitemic women in the current study was small (16 samples), there was no suggestion that adding the third dose increased the level of selection. The previous trials comparing IPTp versus placebo or case management found that women protected by ITNs did not benefit from IPTp to the same degree as

women not using ITNs (Mbaye et al., 2006b, Menendez et al., 2008, Menendez et al., 2007).

In the current study, there was no conclusive indication that ITN use modified the effect of IPTp; the 15% of women who used ITNs benefited equally from the third dose, compared with women who were not protected by ITNs in terms of reductions in malaria and LBW. However, the third dose appeared to be more effective in increasing mean birth weight in the non-ITN users than in the ITN users (P for difference in treatment effect between ITN users and nonusers, .12). Similarly, previous trials have shown IPTp-SP (and other interventions, such as ITNs) to be more effective in first pregnancies (Gamble et al., 2007, Mbaye et al., 2006b, Ter Kuile et al., 2007), yet in this setting, with highly seasonal transmission, gravidity was not found to be a significant effect modifier, and similar reductions in placenta malaria and LBW were observed in the first 2 pregnancies, compared with multigravidae, although the greatest effect on mean birth weight was observed in the first 2 pregnancies. In contrast to the large impact on LBW and preterm delivery (Figure 3.2), the impact on anaemia (haemoglobin level <11 g/dL) was modest and non-significant (APR, 0.89; P =0.14); however, the point estimate is consistent with the summary estimate from the previous trials of 2-dose IPTp and ITNs, which also found an average risk reduction of 10% (Gamble et al., 2007, Ter Kuile et al., 2007).

Our study is limited by the lack of use of a placebo and provision of study drugs by clinicians hired by the study. The study was partially masked, in that none of the laboratory staff or delivery unit staff were aware of the study assignment, but the lack of use of a placebo makes any study more vulnerable to bias. Both groups otherwise received the same antenatal care, and there was no difference in the number of scheduled visits between the arms. The policy implications of this study imply that more frequent dosing would now be appropriate for the control of malaria in pregnancy and its sequellae in areas with low SP resistance in western Africa. Despite the support from many initiatives in sub-Saharan Africa, coverage with 2 doses of SP remains very low, and many women attending ANC receive a single dose of SP only (Van Eijk et al., 2011). The experience has been that single-dosing is a common result of unsuccessfully trying to implement a 2-dose regimen; extending to 3 doses of IPTp-SP may thus be more effective in an operational context, because it counters the risk under dosing mothers (Gill et al.,

2007). The other benefit of more-frequent dosing is this allows for a better integration with existing services as part of focused antenatal care, which consists of 4 scheduled visits, including 3 in the second and third trimester.

These results suggest that malaria in the last few weeks of pregnancy is a major cause of LBW and that the addition of a third dose of SP to the 2-dose IPTp regimen used in most countries in western Africa may have a substantial beneficial impact on public health in the Sahel countries in sub- Saharan Africa, most of which currently have low levels of SP resistance.

Chapter 4: Intermittent preventive therapy for malaria during pregnancy using 2 vs 3 or more doses of sulfadoxine-pyrimethamine and risk of low birth weight in Africa: systematic review and meta-analysis

Chapter 4

4 Intermittent preventive therapy for malaria during pregnancy using 2 vs 3 or more doses of sulfadoxine-pyrimethamine and risk of low birth weight in Africa: systematic review and meta-analysis

4.1 Introduction and background

In areas of stable malaria transmission in sub-Saharan Africa, *Plasmodium falciparum* infection in pregnant women is associated with maternal anaemia and low birth weight (LBW) (<2500 g) (Desai et al., 2007, Steketee et al., 2001, World Health Organization, 2004), especially among primigravida and secundigravida and human immunodeficiency virus (HIV)–infected women (Desai et al., 2007). The World Health Organization (WHO) recommended intermittent preventive therapy during pregnancy, consisting of at least 2 full treatment doses of sulfadoxine-pyrimethamine for HIV-negative women and at least 3 doses for HIV-positive women not receiving cotrimoxazole, administered presumptively in the second and third trimesters at least 1 month apart (WHO/AFRO, 2004, World Health Organization, 2006). Each dose suppresses or clears any existing asymptomatic infections from the placenta and provides up to 6 weeks of post-treatment prophylaxis (White, 2005, WHO/AFRO, 2004). Although the 2-dose regimen provides at most 12 weeks of prophylaxis (Ter Kuile et al., 2007, White, 2005), it has been shown to be effective in reducing LBW (Diakite et al., 2011, Kayentao et al., 2005, Mbaye et al., 2006b, Njagi et al., 2003, Parise et al., 1998, Shulman, 1999, Ter Kuile et al., 2007) and was adopted by 31 of 37 endemic countries in Africa with a policy for intermittent preventive therapy during pregnancy; the remaining countries use a 3-dose or monthly regimen (Van Eijk et al., 2011). Nevertheless, reinfections are common with the 2-dose regimen, especially among women who complete their last dose early in the third trimester (Diakite et al., 2011, Kayentao et al., 2005). A previous meta-analysis of 3 trials confirmed that additional doses of sulfadoxine-pyrimethamine may add benefit over 2 doses among HIV-infected primigravida plus secundigravida (G1-G2 women), but there was insufficient evidence on HIV-negative women or intermittent preventive therapy during pregnancy when used in combination with insecticide-treated nets (Ter Kuile et al., 2007). Furthermore, increasing sulfadoxine-pyrimethamine resistance, which results in a

progressive decrease of the duration of the prophylactic effect (White, 2005), may also require more frequent dosing (Ter Kuile et al., 2007).

The objective of this analysis was to evaluate whether 3 or more doses of intermittent preventive therapy during pregnancy with sulfadoxine-pyrimethamine are associated with higher birth weight or a lower risk of LBW than the 2-dose regimen and to examine whether this is moderated by sulfadoxine-pyrimethamine resistance, HIV status, gravidity, or use of insecticide-treated nets.

4.2 Methods

4.2.1 Eligibility criteria

Study inclusion criteria, outcomes, and methods for the analysis were prespecified in the protocol. Studies had to be quasi-randomized or randomized controlled trials conducted with pregnant women living in sub-Saharan Africa, which compared the 2-dose regimen with sulfadoxine-pyrimethamine with a regimen of intermittent preventive therapy during pregnancy consisting of 3 doses or monthly dosing. Studies or study groups that combined sulfadoxine-pyrimethamine with other antimalarial drugs, such as artemisinin derivatives or azithromycin, or other interventions, such as screening for malaria, were excluded. Use of mosquito nets was not an exclusion criterion. Trial inclusion was unrestricted by gravida group, HIV status, and type of outcomes reported.

4.2.2 Study selection

Studies were identified by searching PubMed, SCOPUS, ISI Web of Knowledge, EMBASE, LILACS, Cochrane CENTRAL, the Malaria in Pregnancy Library (van Eijk et al., 2012), WHO's International Clinical Trials Registry Platform, and the Cochrane Central Register of Controlled Trials from their inception to December 11, 2012, without language restrictions; scanning reference lists of articles; and consultation with experts in the field (see search criteria and PubMed Search string bellow and (Figure 4.1) .

For trial selection, 2 authors (K.K. and A.M.v.E.) independently screened and assessed trials for eligibility and final inclusion in the analysis in a standardized manner. Disagreement between reviewers was resolved through consensus after discussion and consultation with the senior author (F.O.t.K.).

Chapter 4

Search Criteria

We searched PubMed, SCOPUS, ISI web for knowledge, Cochrane CENTRAL, and the Malaria in Pregnancy Library (van Eijk et al., 2012), and WHO's International Clinical Trials Registry Platform and the Cochrane Central Register of Controlled Trials

PubMed Search String

The search terms were '(Malaria AND pregnan* AND intermittent AND (prevent* OR prophyla* OR presumpt* OR chemoprevent* OR chemoprophyla* OR IPT*) AND (sulfadoxine OR sulphadoxine OR pyrimethamine OR SP)' with the results unrestricted by language, publication date or publication status.

PubMed Search String		
PubMed Mesh String	**Search name**	
1	Malaria	Malaria
2	Pregnancy	Pregnan*
3	Intermittent	Intermittent
4	Preventions or preventive	Prevent*
5	Prophylaxix	Prophyla*
6	Presumptive	Presumpt*
7	Chemo preventive	Chemoprevent*
8	Chemoprophylaxis	Chemoprophylaxis*
9	IPT	IPT*
10	Sulfadoxine	Sulfadoxine
11	Sulphadoxine	Sulphadoxine
12	Pyrimethamine	Pyrimethamine
13	SP	SP
14	1 &2	
15	14 & 3	
16	4 or 5 or 6 or 7 or 8 or 9	
17	15 & 16	
18	10 or 11 or 12 or 13	
19	18 or 18	

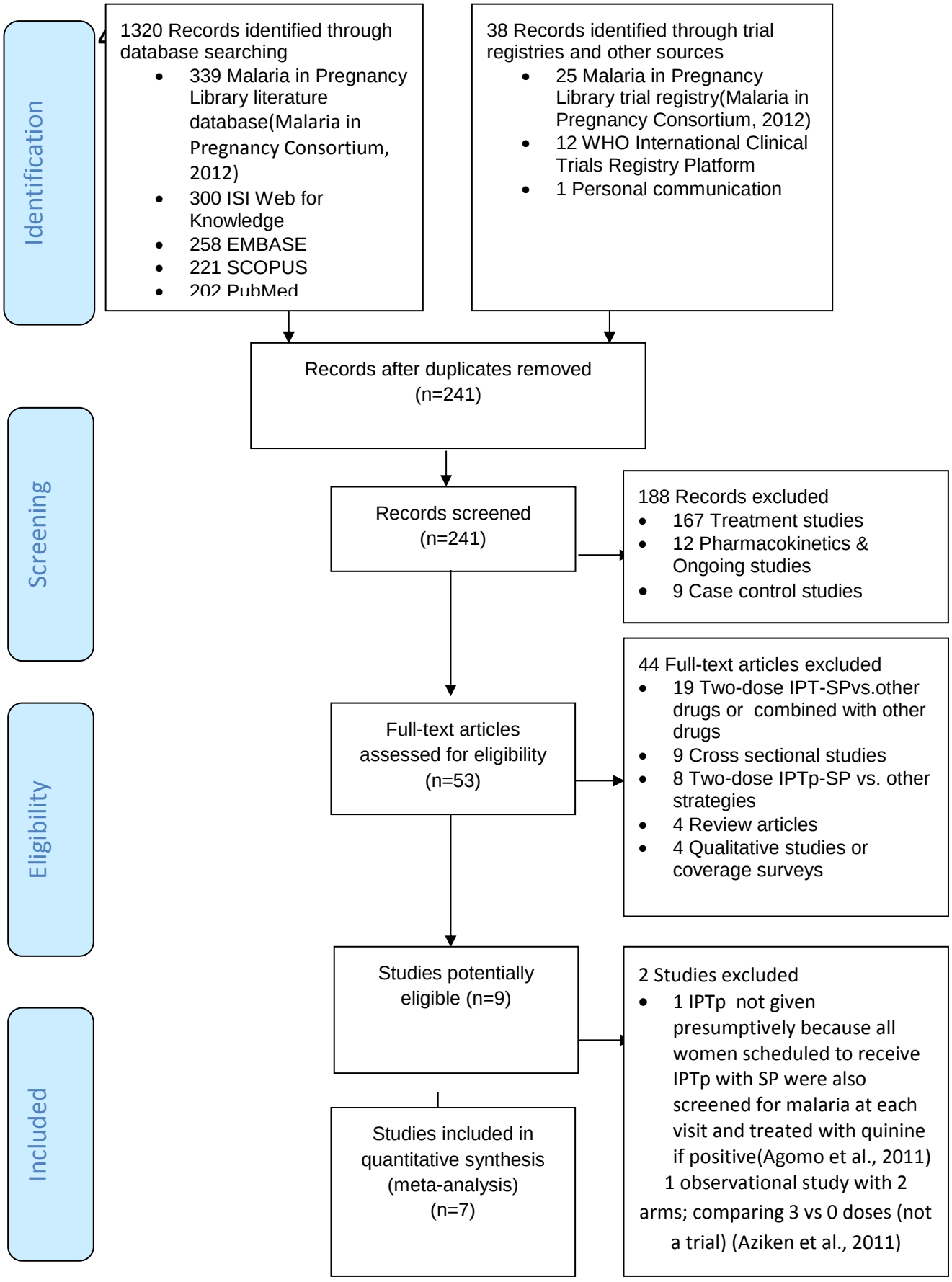


Figure 4.1: PRISMA flow diagram

Notes: Abbreviations: WHO, World Health Organization; n, number; IPTp, Intermittent preventive treatment; SP, sulfadoxine- pyrimethamine; vs, versus. Qualitative studies included studies using focus group discussions or in-depth interviews, coverage surveys included surveys of IPTp uptake in the community.

4.2.3 Data collection and analysis

Data extraction was conducted independently by 2 unblinded investigators (K.K. and A.M.v.E.) using pretested standardized data extraction forms. Authors of primary studies were contacted for missing information or if reported data did not fit the required format. For each study, the following information was extracted: first author, publication year, year of study start and end, study design, randomization procedures, inclusion criteria (eg, any restrictions by gravidity, age, or HIV status), insecticide-treated net or bed net use, folate supplementation and dosage, local malaria transmission, details of study groups, number of women enrolled, and outcomes assessed, including adverse events overall and stratified by subgroup. The Cochrane Collaboration's tool for assessing the risk of bias (Higgins et al., 2011) was used to determine the quality of included trials as low (high risk of bias), high (low risk of bias), or unclear. Uncertainties were resolved by consensus and by contacting the corresponding authors (Collaboration., 2008).

Time and location-matched data on molecular resistance to sulfadoxine-pyrimethamine were obtained from published articles, as described previously (Naidoo and Roper, 2011), and through correspondence with the authors of the trials. The prevalence of the *K540E* mutation in the dihydropteroate synthase (*DHPS*) gene was used as a proxy for the prevalence of the combined dihydrofolate reductase *DHFR* (N51I, C59R, and S108N) */DHPS* (A437G, K540E) quintuple genotype that is strongly associated with treatment failure of sulfadoxine-pyrimethamine (Picot et al., 2009).

4.2.4 Synthesis

The primary outcome measures were LBW and mean birth weight. Secondary outcomes included maternal haemoglobin level, maternal anaemia (haemoglobin level <11 g/dL) and moderate to severe anaemia (defined by the individual trials as haemoglobin level <6, 7, or 8 g/dL) at term or delivery, maternal malaria infection (peripheral blood) at delivery, placental malaria infection (all species), preterm delivery (<37 weeks' gestation), spontaneous miscarriage, stillbirth, and neonatal death (death within 0-27 days in live-born infants). All analyses were stratified a priori by HIV status and gravidity status (G1-

G2 vs ≥G3 pregnancies [multigravida]), with the aim to provide independent subgroup estimates and overall estimates of the pooled data.

We used both random-effects (primary method) and fixed-effects models to calculate the summary relative risks (RRs) for dichotomous outcomes (Mantel-Haenszel) or differences in means for continuous outcomes (inverse variance) and we prespecified that any heterogeneity would be investigated by subgroup analysis. To provide estimates of absolute risk and effect, values for the assumed control-group risk in 2-dose recipients and the corresponding intervention-group risk and 95% CI in ≥3-dose recipients were computed as assumed control-group risk = median risk (expressed per 1000 women) across the included trials in the 2-dose group; corresponding intervention-group risk = assumed control-group risk × RR (95% CI), where the RR was taken from random-effects models (Schunemann, 2008). The absolute risk reduction was calculated as the assumed control-group risk × (1 − RR) and expressed per 1000 women. Similar methods were used with the lower and upper CI of the RR to obtain the 95% CI of the absolute risk reduction. The number needed to treat (NNT) for LBW (the primary end point) was computed as NNT = 1/(assumed control-group risk × [1 − RR]) (Schunemann, 2008). For the continuous end points, the observed median birth weight or haemoglobin concentration in the 2-dose group was reported as the assumed control-group median. The corresponding value in ≥3-dose recipients was expressed as the corresponding intervention-group median and 95% CI, which were computed as the assumed control-group median + mean difference (95% CI).

Heterogeneity was quantified with the I^2 statistic and χ^2 test (Higgins et al., 2003). The Deeks and Higgens method was used to test for heterogeneity between the different summary estimates across subgroups (Deeks, 2010, Holmes et al., 2011) . Publication and small-study bias was assessed by visual inspection of funnel plots and the Harbord test. To evaluate the change in pooled summary estimates for the RR with addition of new evidence, we created cumulative meta-analysis plots (Holmes et al., 2011). Prespecified sensitivity analysis for the primary outcomes was performed by excluding all studies that were scored as low quality for allocation concealment or other sources of bias (Higgins et al., 2011). Further sensitivity analysis was conducted to test the effect of each study on

the pooled estimates and heterogeneity by removing one study at a time from the meta-analysis. We used $P < .05$ to indicate statistical significance (2-sided tests). Data were analyzed with Review Manager version 5.2, GradePro version 3.6, and Stata version 12.

4.3 Results

4.3.1 Studies and outcomes

A total of 241 studies were screened, and 7 trials including a total of 6281 pregnancies were included (Figure 4.1), (Diakite et al., 2011, Filler et al., 2006, Luntamo et al., 2010, Luntamo et al., 2012, MaArthur, 2005, Parise et al., 1998, Valea et al., 2010) one of which was unpublished (MacArthur and Abdulla, 2005) (Table 4. 1). Authors of all primary studies provided further unpublished information where available. Five trials compared monthly sulfadoxine-pyrimethamine against the 2-dose regimen and the remaining 2 compared 3- vs 2-dose intermittent preventive therapy during pregnancy with sulfadoxine-pyrimethamine (Diakite et al., 2011, Valea et al., 2010). Sulfadoxine-pyrimethamine intake was supervised in all trials. Three trials in Kenya and Malawi involved both HIV-infected and uninfected women (Filler et al., 2006, Luntamo et al., 2010, Parise et al., 1998) and 1 trial in Zambia involved HIV-infected women only (Hamer et al., 2007). In 3 other trials, the HIV status was unknown (Diakite et al., 2011, Valea et al., 2010, MacArthur and Abdulla, 2005), 2 of which were from areas with very low HIV prevalence among pregnant women (1% in Burkina Faso and 1.3% in Mali) (Diakite et al., 2011, Valea et al., 2010); results were therefore pooled with those of the HIV-negative women. The third trial from Tanzania (MacArthur and Abdulla, 2005) was conducted in an area with high HIV prevalence and analyzed as a separate “HIV status unknown” stratum. Two of the 7 trials were considered of low quality (Figure 4.2), including a trial in Burkina Faso, in which two-thirds of participants did not receive the intended regimen (Valea et al., 2010). The other study was a quasi-randomized trial (Parise et al., 1998) conducted before the introduction of the Consolidated Standards of Reporting Trials (CONSORT) guidelines for clinical trials (Begg et al., 1996) (Table 4. 1).

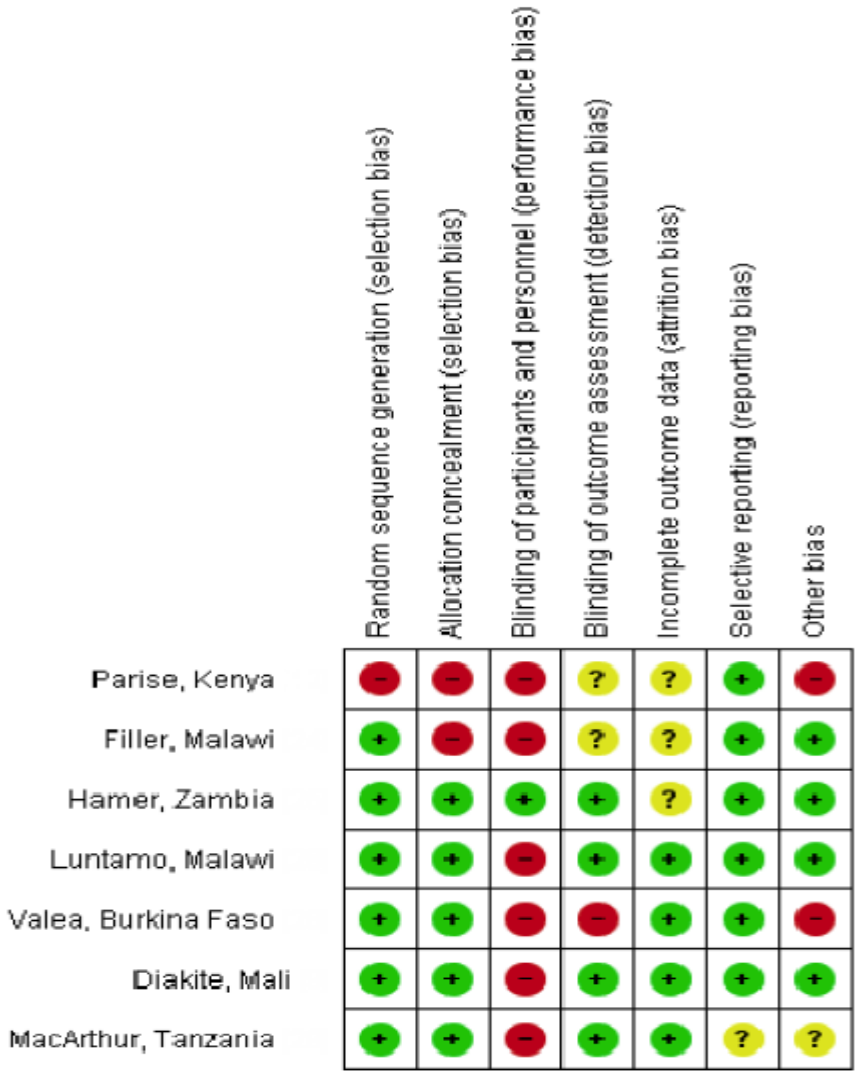


Figure 4.2 : Risk of bias assessment representing the authors' judgements about each risk of bias item for each included study across the domains

Figure note: Graph represents the risk of bias assessment using criteria described by Higgins et al.(Higgins et al., 2011). Two studies were classified as low quality, including the study by Valea et al which had a high risk of 'other' bias because the study was designed to provide all IPT courses by study staff in the home of the participants. Because of logistical constraints, only a minority received the number of intended doses with marked differences in missed doses between the study arms (only 23% and 41% of the women in the 3+, - and 2-dose arms received a third and second dose, respectively).

Chapter 4

Table 4. 1: Characteristics of included Trials

		Parise	Filler	Hamer	Luntamo	Valea	Diakite	MacArthur
Country		Kenya	Malawi	Zambia	Malawi	Burkina Faso	Mal	Tanzania
Year Published		1998	2006	2007	2010	2010	2011	Unpubl.[a]
Study years		1994-1996	2002-2005	2003-2004	2003-2006	2006-2008	2006-2008	2003-2006
Gravidae		G1-G2	G1-G2	All	All	All	All	G1-G2
Number of women	3+dose	661	351	224	441	656	413	400
	2-dose	680	347	232	436	640	401	399
	Total (G1-G2)	1341 (1341)	698 (698)	456 (251)	877 (371)	1296 (536)	814 (339)	799 (799)
Intervention arm regimen		Monthly	Monthly	Monthly	Monthly	3-dose[b]	3-dose	Monthly
Number of doses in 3+ arm, median (range)		3 (1-5)	5 (1-5)	4 (1-6)	4 (1-6)	2 (1-3)[b]	3 (1-3)	3 (1-5)
Median Number of ANC Visits	3+dose	Designed to be equal-[c]	Designed to be equal-[c]	Designed to be equal-[c]	4.4 (1-9)[d]	4 (1-7)	3 (1-6)	4 (1-6)
	2-dose				4.4 (1-9)[d]	4 (1-6)	3 (1-6)	3 (1-7)
HIV status		Pos+Neg	Pos+Neg	Pos only	Pos+Neg[e]	All[f]	All[f]	Pos+Neg[g]
Malaria Transmission[h]		Holoendemic	Holoendemic	Holoendemic[i]	Holoendemic	Hyperendemic	Hyperendemic	Holoendemic
Entomological Inoculation rate (EIR)/yr[j]		60-300	18-27	NA	NA	NA	NA	367
SP resistance N (% *dhps* K540E)[k]		77 (14%)	76 (96%)	24 (46%)	88 (86%)	80 (0%)	9 (0%)	120 (46%)
Folic Acid dose in mg/day		5	0.5	5	0.25	0.4	0.4	0.4
Bednet coverage, N (%)		148 (11)	698 (15)	456 (25)	1320 (60)	288 (14)	814 (17)	799 (37)
Random Sequence Generation		Not random	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate
Sequence Allocation		By day of visit	Inadequate	Adequate	Adequate	Adequate	Adequate	Adequate
Open label /Placebo controlled		Open Label	Open Label	Placebo	Open Label	Open Label	Open Label	Open Label
Assessor blinding birthweight		No	No	Yes	Yes	No	Yes	Yes
Loss-to-follow-up (%)		478 (36)	143 (22)	68 (15)	86 (10)	259 (20)	73 (9)	56 (7)

Abbreviations: HIV, Human Immunodeficiency Virus; SP, Sulfadoxine-Pyrimethamine; *dhps*, dihydropteroate synthase; G1-G2, first and second pregnancies; G3+, 2 or more previous pregnancies; ANC, Antenatal Clinic; Pos, HIV-Positive and Neg, HIV-Negative. NA, Not available

[a] All information was provided by two of the co-authors (AM, JRM)

[b] Drug administration was provided as directly observed therapy in the home environment. However, because of logistical reasons only 149 (23%) of the women in the 3-dose group received the third sulfadoxine-pyrimethamine dose and only 261 (41%) in the 2-dose group received a second sulfadoxine-pyrimethamine dose.

[c] Actual number of visits not reported, but the studies were designed to have identical antenatal care schedules in both arms

[d] Mean (range) instead of the median

[e] The HIV negative group includes 82 women (42 in the 3+dose group) with unknown/undetermined HIV status.
[f] HIV screening and testing was not conducted, but the HIV prevalence in the general antenatal clinic population were 1.0% and 1.3% in the study sites in Burkina Fasoand Malirespectively.
[g] HIV screening and testing was conducted, but HIV tests results were not available.
[h] Holoendemic: transmission occurs all year long; Hyperendemic: intense, but with periods of no transmission during dry season.
[i] Transmission during the study period was reported to be lower than usual as described as 'mild malaria transmission'.
[J] The Entomological inoculation rate (EIR) is a measure of malaria transmission intensity and is the number of infectious bites per person per unit time (usually expressed per year). It is the product of the human biting rate and the sporozoite rate.
[k] sulfadoxine-pyrimethamine resistance data matched for time and location (<=100km) and defined as the proportion of symptomatic children less than 5 or 12 years old carrying *dhps* K540E mutations for sulfadoxine-pyrimethamine resistance, except for the study by Diakite in Mali and Luntamo in Malawi, which were based on samples from women attending antenatal care prior to their first dose of sulfadoxine-pyrimethamine.

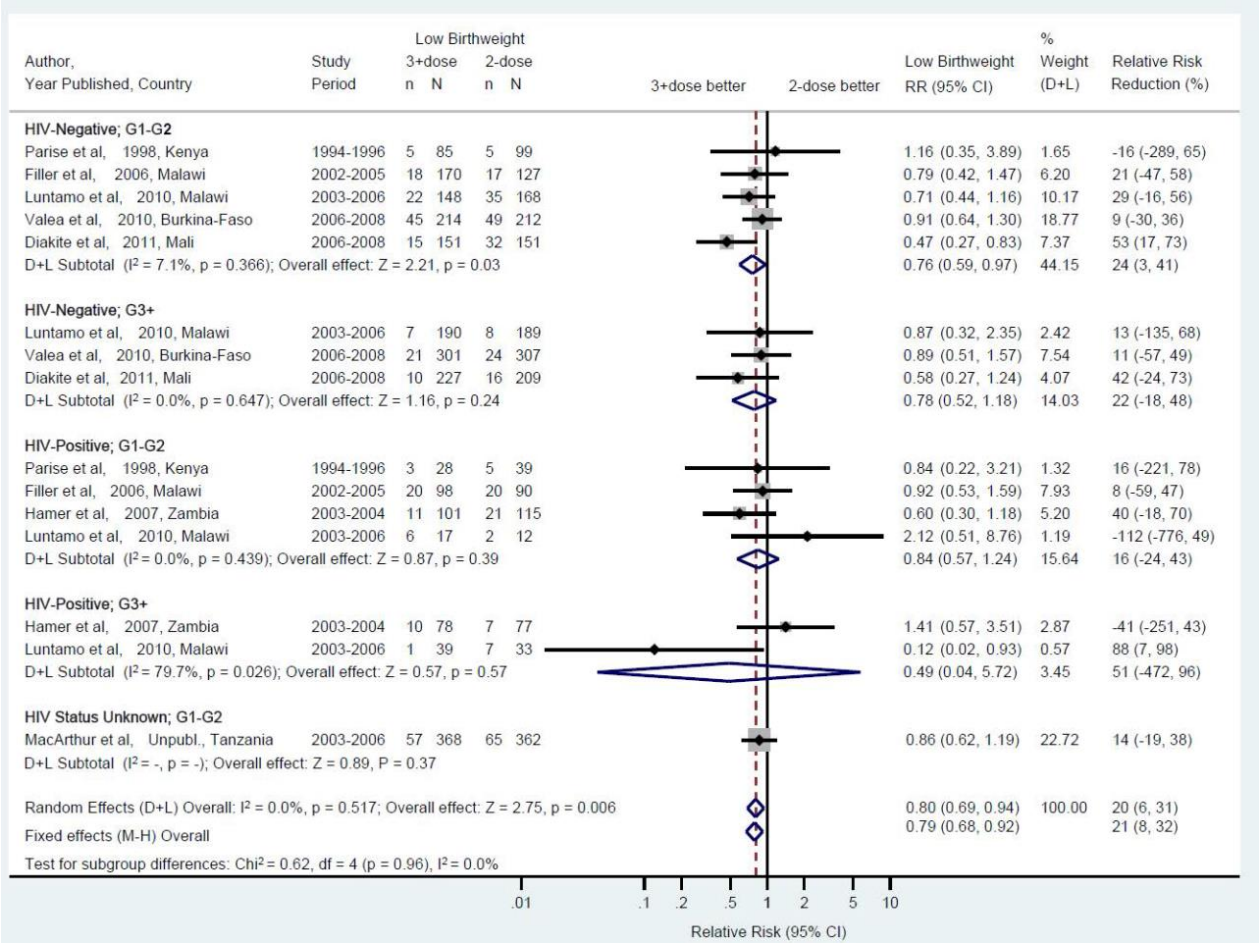


Figure 4.3: Meta-analysis of the risk of low birthweight in trials comparing the 2-dose vs 3+ doses of IPTp with sulfadoxine-pyrimethamine

Figure note: HIV, human immunodeficiency virus; G1-G2, first and second pregnancies; G3+, 2 or more previous pregnancies; n, number of events; N, Total number of women; RR, relative risk; CI, confidence interval; D+L, Dersimonian-Laird method for random effects models; M-H, Mantel-Haenszel method for fixed effects models. P-values following the I^2 statistics represent the Chi-square test for heterogeneity. Weights are from random effects analysis. Data-marker sizes indicate the weight applied to each study using random-effects meta-analysis. Diamonds represent summary effect of studies.

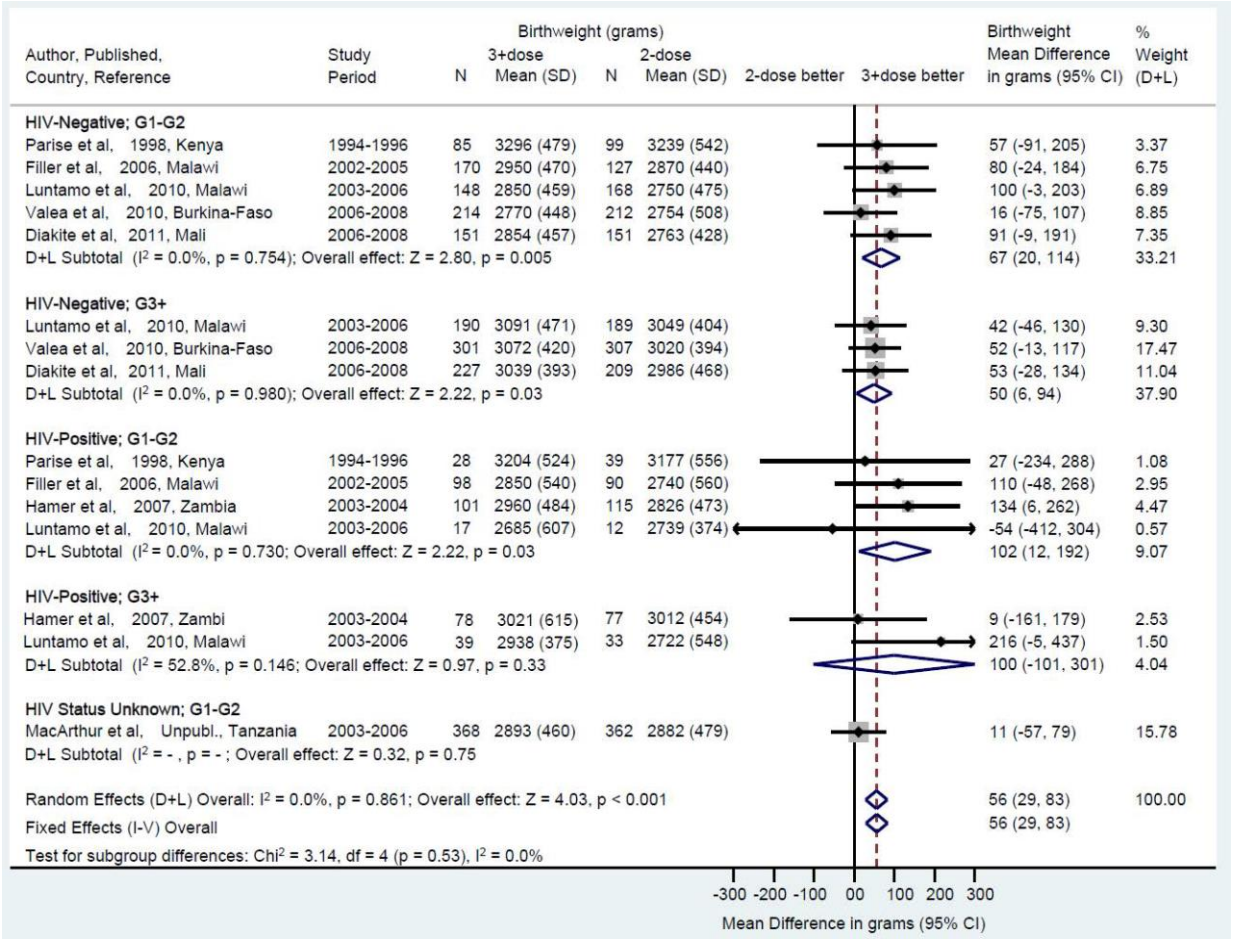


Figure 4. 4: Meta-analysis of mean birthweight in 7 trials comparing the 2-dose vs 3+ doses of IPTp with sulfadoxine-pyrimethamine

Figure note: HIV, human immunodeficiency virus status; *dhps*, dihydropteroate synthase; G1-G2, first and second pregnancies; G3+, 2 or more previous pregnancies; n, number of events; N, sample size; RR, relative risk; CI, confidence interval; D+L, Dersimonian-Laird method for random effects models; I-V, Inverse-Variance method used in the fixed effects models. P-values following the I^2 statistics represent the chi-square test for heterogeneity. Weights are from random effects analysis. Data-marker sizes indicate the weight applied to each study using random-effects meta-analysis. Diamonds represent summary effect of studies.

4.3.2 Primary outcomes: Birth weight

Women in the ≥3-dose group had fewer infants with LBW (random-effects model RR=0.80; 95% CI, 0.69-0.94; *P*=0.006; I^2=0%) (Figure 4. 3), corresponding to an RR reduction (RR reduction = 100% × [1 − RR]) of 20% (95% CI, 6-31). The absolute risk reduction was 33 per 1000 women (95% CI, 10-52), from a median risk of 167 per 1000 in the 2-dose group (assumed control-group risk) to 134 per 1000 in the ≥3-dose recipients (NNT = 31). The median birth weight in the 2-dose group was 2870 g (range, 2722-3239 g) and on average 56 g (95% CI, 29-83 g) higher in the ≥3-dose group (Figure 4. 4 & Table 4. 2). Analyses by gravida and HIV subgroup showed that the mean difference in birth weight was statistically significant in HIV-negative women (random-effects mean difference = 58 g; 95% CI, 26-90 g), HIV-positive women (mean difference = 97 g; 95% CI, 22-172) (Table 4. 2), G1-G2 women (mean difference = 57 g; 95% CI, 22-93 g), (Table 4. 3) , and multigravida (mean difference = 53 g; 95% CI, 11-95 g) (Table 4. 4) (between-subgroup difference, I^2 = 0%; *P* = .53) . The RR estimates for LBW, however, were significant only in HIV-negative women (RR = 0.77 [95% CI, 0.63-0.94] (Table 4. 2); assumed control-group risk = 106 per 1000; absolute risk reduction = 24 per 1000 [95% CI, 6-39]; NNT = 42) and G1-G2 women (RR = 0.80 [95% CI, 0.68-0.95] ; assumed control-group risk = 181 per 1000; absolute risk reduction = 36 per 1000 [95% CI, 9-58]; NNT = 28) but not in HIV-positive women (RR = 0.86 [95% CI, 0.53-1.39] (Table 4. 2); assumed control-group risk = 175 per 1000; absolute risk reduction = 24 per 1000 [95% CI, −68 to 82]; NNT = 42) or multigravida (RR = 0.79 [95% CI, 0.49-1.27], (Table 4. 4); assumed control-group risk = 78 per 1000; absolute risk reduction = 16 per 1000 [95% CI, −21 to 40]; NNT = 63). The difference in the RR estimates between the subgroups was not significant (between-subgroup difference (I2=0%; P=0.96) (Figure 4.3). The results of fixed-effects models overall and by gravidity or HIV groups were mostly identical or very similar (Table 4. 5)

Table 4. 2:: Random effects meta-analysis of trials comparing the 2-dose vs 3+ doses of IPTp with sulfadoxine-pyrimethamine by HIV status

		2- dose			3+dose			Random effects model			
	No. of studies	No. Events	Total No.	ACR per 1000 or ACM (Range) [a]	No. Events	Total No.	CIR per 1000 or CIM (95% CI) [a]	Relative Risk (95% CI) [b]	ARR per 1000 or Mean Difference (95% CI) [c]	P-Value [b]	I^2
Primary Endpoints											
Low Birthweight											
HIV-positive	4	62	366	175 (91-222)	51	361	151 (93-243)	0.86 (0.53-1.39)	24 (-68-82)	.54	33%
HIV-negative	5	186	1462	106 (42-231)	143	1486	82 (67-100)	0.77 (0.63-0.94)	24 (6-39)	.01	0%
Unknown	1	65	362	180[d]	57	368	155 (112-214)	0.86 (0.62-1.19)	25 (-34-68)	.37	-[d]
Overall	7	313	2190	167 (42-231)	251	2215	134 (115-157)	0.80 (0.69-0.94)	33 (10-52)	.006	0%
Birthweight, grams											
HIV-positive	4		366	2783 (2722-3177)		361	2880 (2805-2955)		97 (22-172)	.01	0%
HIV-negative	5		1462	2928 (2750-3239)		1486	2986 (2954-3018)		58 (26-90)	<.001	0%
Unknown	1		362	2882[d]		368	2893 (2825-2961)		11 (-57-79)	.75	-[d]
Overall	7		2190	2870 (2722-3239)		2215	2926 (2899-2953)		56 (29-83)	<.001	0%
Secondary Endpoints											
Maternal haemoglobin, g/dL											
HIV-positive	4		349	11.0 (9.7-11.4)		327	11.1 (10.9-11.4)		0.11 (-0.15-0.37)	.40	0%
HIV-negative	5		1395	10.8 (10.2-11.6)		1461	11.0 (10.8-11.1)		0.15 (0.04-0.26)	.009	0%
Unknown	1		344	11.1[d]		340	11.1 (10.8-11.4)		0.00 (-0.31-0.31)	1	-[d]
Overall	7		2088	10.9 (9.7-11.6)		2128	11.0 (10.9-11.1)		0.13 (0.03-0.22)	.009	0%
Maternal anaemia (<11g/dL)											
HIV-positive	4	214	349	582 (333-795)	190	327	559 (506-623)	0.96 (0.87-1.07)	23 (-41-76)	.51	0%
HIV-negative	5	665	1395	473 (269-660)	682	1461	459 (426-492)	0.97 (0.90-1.04)	14 (-19-47)	.37	0%
Unknown	1	175	344	509[d]	152	340	448 (382-524)	0.88 (0.75-1.03)	61 (-15-127)	.11	-[d]
Overall	7	1054	2088	509 (269-795)	1024	2128	484 (458-514)	0.95 (0.90-1.01)	25 (-5-51)	.10	0%
Moderate-severe maternal anaemia (<8 or <=7 or <=6 g/dL)											
HIV-positive	2	7	124	00 (00-65)	3	135	00 (00-00)	0.60 (0.06-5.85)	0 (0-0)	.66	48%
HIV-negative	4	38	1296	38 (09-63)	27	1376	27 (14-52)	0.70 (0.36-1.36)	11 (-14-24)	.29	34%
Unknown	2[e]	25	776	32 (30-35)	21	771	27 (15-48)	0.85 (0.48-1.50)	5 (-16-17)	.57	0%
Overall	6	70	2196	34 (00-65)	51	2282	25 (16-38)	0.73 (0.48-1.11)	9 (-4-18)	.14	15%

(Table 4. 2. continued)

		2- dose			3+dose			Random effects model			
	No. of studies	No. Events	Total No.	ACR per 1000 or ACM (Range) [a]	No. Events	Total No.	CIR per 1000 or CIM (95% CI) [a]	Relative Risk (95% CI) [b]	ARR per 1000 or Mean Difference (95% CI) [c]	P-Value [b]	I^2
Maternal parasitemia											
HIV-positive	4	51	338	112 (00-359)	13	328	29 (17-52)	0.26 (0.15-0.46)	83 (60-95)	<.001	0%
HIV-negative	5	265	1407	104 (31-350)	234	1445	89 (77-105)	0.86 (0.74-1.01)	15 (-1-27)	.06	0%
Unknown	1	7	351	20 [d]	2	349	06 (01-27)	0.29 (0.06-1.37)	14 (-7-19)	.12	- [d]
Overall	7	323	2096	92 (00-359)	249	2122	63 (48-82)	0.68 (0.52-0.89)	29 (10-44)	.005	47%
Placental malaria											
HIV-positive	4	39	338	102 (00-256)	14	320	39 (21-70)	0.38 (0.21-0.69)	63 (32-81)	.001	0%
HIV-negative	4	82	753	67 (00-201)	47	782	38 (26-55)	0.57 (0.39-0.82)	29 (12-41)	.003	9%
Unknown	1	7	345	20 [d]	4	344	11 (03-39)	0.57 (0.17-1.94)	9 (-19-17)	.37	[d]
Overall	6	128	1436	63 (00-256)	65	1446	32 (24-43)	0.51 (0.38-0.68)	31 (20-39)	<.001	0%
Preterm delivery											
HIV-positive	3	130	331	306 (46-655)	113	340	278 (211-370)	0.91 (0.69-1.21)	28 (-64-95)	.51	32%
HIV-negative	4	209	1479	107 (16-248)	191	1554	93 (72-122)	0.87 (0.67-1.14)	14 (-15-35)	.32	41%
Unknown	2 [e]	51	769	61 (21-102)	66	777	78 (55-111)	1.28 (0.90-1.82)	-17 (-50-6)	.17	1%
Overall	7	390	2579	122 (16-655)	370	2671	116 (98-137)	0.95 (0.80-1.12)	6 (-15-24)	.52	35%
Miscarriage											
HIV-positive	2	3	147	00 (00-30)	5	171	00 (00-00)	1.54 (0.38-6.28)	0 (0-0)	.55	- [d]
HIV-negative	4	19	1515	00 (00-29)	28	1587	00 (00-00)	1.31 (0.64-2.70)	0 (0-0)	.46	20%
Unknown	2 [e]	5	809	06 (00-12)	9	809	11 (04-32)	1.80 (0.61-5.34)	-5 (-26-2)	.29	- [d]
Overall	6	27	2471	00 (00-30)	42	2567	00 (00-00)	1.43 (0.88-2.33)	0 (0-0)	.15	0%
Stillbirth											
HIV-positive	3	11	352	40 (00-56)	8	362	27 (11-70)	0.68 (0.27-1.74)	13 (-30-29)	.43	0%
HIV-negative	4	44	1515	30 (15-53)	60	1587	40 (27-59)	1.33 (0.90-1.95)	-10 (-29-3)	.15	0%
Unknown	2 [e]	24	809	30 (25-34)	24	809	29 (13-68)	0.97 (0.42-2.27)	1 (-38-17)	.95	54%
Overall	7	79	2676	30 (00-56)	92	2758	34 (26-46)	1.14 (0.85-1.55)	-4 (-16-4)	.38	0%
Neonatal death [f]											
HIV-positive	2	10	137	77 (29-167)	6	160	39 (14-112)	0.51 (0.18-1.45)	38 (-35-63)	.21	0%
HIV-negative	4	25	1472	19 (08-31)	32	1549	23 (13-39)	1.19 (0.69-2.05)	-4 (-20-6)	.54	0%
Unknown HIV status	2 [e]	14	796	18 (14-22)	7	800	08 (02-33)	0.47 (0.12-1.84)	10 (-15-16)	.28	37%
Overall	6	49	2405	21 (08-167)	45	2509	18 (12-28)	0.88 (0.57-1.35)	3 (-7-9)	.55	0%

Abbreviation: No, number; 3+dose, monthly or 3 doses of sulfadoxine-pyrimethamine; ACR, Assumed Control-Group Risk; ACM, Assumed Control-Group Median; CIR Corresponding Intervention-Group Risk; CIM, Corresponding Intervention-Group Median; ARR, Absolute Risk Reduction (risk difference), HIV,

Human Immunodeficiency Virus; RR, summary Relative risk adjusted by stratification for gravidity group (G1-G2, G3+) obtained from random effect models ; CI, Confidence Interval; g/dl, gram per deciliter; I^2, statistical test for heterogeneity across trials.

[a] ACR represents the observed median risk (expressed per 1000 women) across the trials in the 2-dose arm; the CIR (and its 95% CI) is based on the assumed risk in 3+dose recipients computed as ACR x RR (Schunemann, 2008). For the 2 continuous endpoints, the ACM represents the median birthweight or haemoglobin concentration in the 2-dose arm (the range is provided to illustrate high and low risk populations); The CIM values were computed as the ACM + Mean Difference (95% CI).

[b] Effect size, 95% confidence intervals and P-values for the overall effect (last rows) and for each HIV-status subgroup were obtained from random effects models and are adjusted for gravidity group (all estimates [G1-G2, G3+]) plus for HIV status (for last rows representing the overall effect), by using the independent subgroups as the unit of analysis.

[c] The absolute risk reduction (ARR) was calculated as the ACRx(1-RR) and expressed per 1000 women.

[d] Range or heterogeneity cannot be estimated, either because the data contains only a single trial in the subgroup, or no events occurred in 1 of the 2 included studies ((Parise et al., 1998)

[e] Results for the study by Parise et al in Kenya were not reported by HIV status for these endpoints

[f] Death of a live-born baby within the first 28 days of life. One study assessed early neonatal death only (death within 7 days of life) .(MacArthur et al., 2007).

Table 4. 3: Random effects meta-analysis of trials comparing the 2-dose vs 3+ doses of IPTp with sulfadoxine-pyrimethamine by HIV status among first and second pregnancies

		2- dose			3+dose			Random effects model			
	No. of studies	No. Events	Total No.	ACR[a] per 1000 or ACM[a] (Range)	No. Events	Total No.	CIR[a] per 1000 or CIM[a] (95% CI)	Relative Risk (95% CI)[b]	ARR per 1000 or Mean Difference (95% CI)[c]	P-Value[b]	I^2
Primary Endpoints											
Low Birthweight											
HIV-positive	4	48	256	175 (128-222)	40	244	147 (100-217)	0.84 (0.57-1.24)	28 (-42-75)	.39	0%
HIV-negative	5	138	757	208 (51-231)	105	768	158 (123-202)	0.76 (0.59-0.97)	50 (6-85)	.03	7%
Unknown	1	65	362	180[d]	57	368	155 (112-214)	0.86 (0.62-1.19)	25 (-34-68)	.37	-[d]
Overall	7	251	1375	181 (51-231)	202	1380	145 (123-172)	0.80 (0.68-0.95)	36 (9-58)	.01	0%
Birthweight, grams											
HIV-positive	4		256	2783 (2739-3177)		244	2885 (2795-2975)		102 (12-192)	.03	0%
HIV-negative	5		757	2763 (2750-3239)		768	2830 (2783-2877)		67 (20-114)	.005	0%
Unknown	1		362	2882[d]		368	2893 (2825-2961)		11 (-57-79)	.75	-[d]
Overall	7		1375	2795 (2739-3239)		1380	2852 (2817-2888)		57 (22-93)	.002	0%
Secondary Endpoints											
Maternal haemoglobin, g/dL											
HIV-positive	4		259	10.5 (9.7-11.2)		238	10.6 (10.3-10.9)		0.08 (-0.21-0.38	.58	0%
HIV-negative	5		737	10.4 (10.2-11.6)		793	10.6 (10.5-10.8)		0.24 (0.08-0.39)	.003	0%
Unknown	1		344	11.1[d]		340	11.1 (10.8-11.4)		0.00 (-0.31-0.31	1	-[d]
Overall	7		1340	10.6 (9.7-11.6)		1371	10.8 (10.6-10.9)		0.17 (0.04-0.30)	.008	0%
Maternal anaemia (<11g/dL)											
HIV-positive	4	172	259	651 (333-795)	152	238	631 (566-710)	0.97 (0.87-1.09)	20 (-59-85)	.60	0%
HIV-negative	5	381	737	522 (269-660)	396	793	496 (454-548)	0.95 (0.87-1.05)	26 (-26-68)	.32	0%
Unknown	1	175	344	509[d]	152	340	448 (382-524)	0.88 (0.75-1.03)	61 (-15-127)	.11	-[d]
Overall	7	728	1340	525 (269-795)	700	1371	499 (462-530)	0.95 (0.88-1.01)	26 (-5-63)	.09	0%
Moderate-severe maternal anaemia (<8 or <=7 or <=6 g/dL)											
HIV-positive	2	7	113	3.3 (0.0-6.5)	2	124	09 (02-41)	0.26 (0.06-1.23)	24 (-8-31)	.09	-[d]
HIV-negative	4	25	638	3.8 (3.4-4.3)	12	708	16 (06-43)	0.43 (0.17-1.13)	22 (-5-32)	.09	37%
Unknown	2[e]	25	776	3.2 (3.0-3.5)	21	771	27 (15-48)	0.85 (0.48-1.50)	5 (-16-17)	.57	0%
Overall	6	57	1527	3.6 (0.0-6.5)	35	1603	22 (13-36)	0.60 (0.36-0.99)	14 (0-23)	.045	20%

Table 4.3
(continued)

	2- dose				3+dose			Random effects model			
	No. of studies	No. Events	Total No.	ACR[a] per 1000 or ACM[a] (Range)	No. Events	Total No.	CIR[a] per 1000 or CIM[a] (95% CI)	Relative Risk (95% CI)[b]	ARR per 1000 or Mean Difference (95% CI)[c]	P-Value[b]	I^2
Maternal parasitemia											
HIV-positive	4	50	250	161 (80-359)	12	234	40 (23-71)	0.25 (0.14-0.44)	121 (90-138)	<.001	0%
HIV-negative	5	166	731	117 (65-350)	138	770	95 (77-116)	0.81 (0.66-0.99)	22 (1-40)	.04	5%
Unknown	1	7	351	20[d]	2	349	06 (01-27)	0.29 (0.06-1.37)	14 (-7-19)	.12	-[d]
Overall	7	223	1332	130 (20-359)	152	1353	70 (48-104)	0.54 (0.37-0.80)	60 (26-82)	.002	56%
Placental malaria											
HIV-positive	4	38	253	179 (61-256)	12	229	61 (34-113)	0.34 (0.19-0.63)	118 (66-145)	<.001	0%
HIV-negative	4	50	463	67 (39-201)	30	492	41 (23-71)	0.61 (0.35-1.06)	26 (-4-44)	.08	23%
Unknown H	1	7	345	20[d]	4	344	11 (03-39)	0.57 (0.17-1.94)	9 (-19-17)	.37	-[d]
Overall	6	95	1061	71 (20-256)	46	1065	35 (25-50)	0.50 (0.35-0.70)	36 (21-46)	<.001	0%
Preterm delivery											
HIV-positive	3	83	215	250 (46-655)	68	216	228 (185-280)	0.91 (0.74-1.12)	22 (-30-65)	.37	0%
HIV-negative	4	124	703	176 (16-248)	108	750	150 (109-202)	0.85 (0.62-1.15)	26 (-26-67)	.29	26%
Unknown	2[e]	51	769	61 (21-102)	66	777	78 (55-111)	1.28 (0.90-1.82)	-17 (-50-6)	.17	1%
Overall	7	258	1687	107 (16-655)	242	1743	102 (87-119)	0.95 (0.81-1.11)	5 (-12-20)	.52	7%
Miscarriage											
HIV-positive	2	3	111	15 (00-30)	5	128	23 (06-94)	1.54 (0.38-6.28)	-8 (-79-9)	.55	-[e]
HIV-negative	4	9	723	08 (00-29)	19	775	14 (05-40)	1.70 (0.57-5.05)	-6 (-32-3)	.34	19%
Unknown	2[e]	5	809	06 (00-12)	9	809	11 (04-32)	1.80 (0.61-5.34)	-5 (-26-2)	.29	-[e]
Overall	6	17	1643	06 (00-30)	33	1712	11 (06-19)	1.75 (0.97-3.13)	-5 (-13-0)	.06	0%
Stillbirth											
HIV-positive	3	4	111	20 (00-40)	6	128	23 (07-78)	1.13 (0.33-3.89)	-3 (-58-13)	.84	0%
HIV-negative	4	24	723	33 (15-53)	31	775	42 (25-71)	1.27 (0.75-2.15)	-9 (-38-8)	.37	0%
Unknown	2[e]	24	809	30 (25-34)	24	809	29 (13-68)	0.97 (0.42-2.27)	1 (-38-17)	.95	54%
Overall	7	52	1643	30 (00-53)	61	1712	34 (24-49)	1.14 (0.79-1.65)	-4 (-19-6)	.48	0%

Neonatal death [f]									Table 4.3 (continued)		
HIV-positive	2	9	103	122 (77-167)	4	117	46 (15-151)	0.38 (0.12-1.24)	76 (-29-107)	.11	0%
HIV-negative	4	15	701	21 (13-31)	18	747	24 (12-48)	1.13 (0.55-2.29)	-3 (-27-9)	.74	0%
Unknown	2[e]	14	796	18 (14-22)	7	800	08 (02-33)	0.47 (0.12-1.84)	10 (-15-16)	.28	37%
Overall	6	38	1600	22 (13-167)	29	1664	16 (10-27)	0.74 (0.45-1.24)	6 (-5-12)	.26	0%

Abbreviation: No, number; 3+dose, monthly or 3 doses of sulfadoxine-pyrimethamine; ACR, Assumed Control-Group Risk; ACM, Assumed Control-Group Median; CIR Corresponding Intervention-Group Risk; CIM, Corresponding Intervention-Group Median; ARR, Absolute Risk Reduction (risk difference), HIV, Human Immunodeficiency Virus; RR, summary Relative risk adjusted by stratification for gravidity group (G1-G2, G3+) obtained from random effect models ; CI, Confidence Interval; g/dl, gram per deciliter; I^2, statistical test for heterogeneity across trials.

[a] Assumed Control-Group Risks represents the median risk (expressed per 1000 women) across the trials in the 2-dose arm; the Corresponding Intervention-Group Risk (and its 95% CI) is based on the assumed risk in 3+dose recipients computed as ACR x RR.(Schünemann et al., 2008) For the 2 continuous endpoints, the assumed Control-Group Median represents the median birthweight or haemoglobin concentration in the 2-dose arm (the range is provided to illustrate high and low risk populations); The corresponding Intervention-Group values were computed as the ACM + Mean Difference (95% CI).

[b] Effect size, 95% confidence intervals and P-values for the overall effect (last rows) and for each HIV-status subgroup were obtained from random effects models and are adjusted for HIV status (for last rows representing the overall effect), by using the independent subgroups as the unit of analysis.

[c] The absolute risk reduction (ARR) was calculated as the ACRx(1-RR) and expressed per 1000 women.

[d] The range or heterogeneity cannot be estimated, either because the data contains only a single trial in the subgroup, or no events occurred in 1 of the 2 included studies

[e] Results for the study by Parise et al in Kenya were not reported by HIV status for these endpoints

[f] Death of a live-born baby within the first 28 days of life. One study assessed early neonatal death only (death within 7 days of life).(MacArthur et al., 2007)

Table 4. 4: Random effects meta-analysis of trials comparing the 2-dose vs 3+ doses of IPTp with sulfadoxine-pyrimethamine by HIV status among multigravidae (G3+)

		2- dose			3+dose			Random effects model			
	No. of studies	No. Events	Total No.	ACR[a] per 1000 or ACM[b] (Range)	No. Events	Total No.	CIR[a] per 1000 or CIM[b] (95% CI)	Relative Risk (95% CI)[c]	ARR per 1000 or Mean Difference (95% CI)[c]	P-Value[c]	I^2
Primary Endpoints											
Low Birthweight											
HIV-positive	2	14	110	152 (91-212)	11	117	74 (06-869)	0.49 (0.04-5.72)	78 (-717-146)	.57	80%
HIV-negative	3	48	705	77 (42-78)	38	718	60 (40-91)	0.78 (0.52-1.18)	17 (-14-37)	.24	0%
Overall	4	62	815	78 (42-212)	49	835	62 (38-99)	0.79 (0.49-1.27)	16 (-21-40)	.33	30%
Birthweight, grams											
HIV-positive	2		110	2867 (2722-3012)		117	2967 (2766-3168)		100 (-101-301)	.33	53%
HIV-negative	3		705	3020 (2986-3049)		718	3070 (3026-3114)		50 (6-94)	.03	0%
Overall	4		815	3012 (2722-3049)		835	3065 (3023-3107)		53 (11-95)	.01	0%
Secondary Endpoints											
Maternal haemoglobin, g/dL											
HIV-positive	2		90	11.2 (10.9-11.4)		89	11.4 (10.9-11.9)		0.20 (-0.33-0.73	.46	0%
HIV-negative	3		658	11.1 (10.8-11.5)		668	11.2 (11.0-11.3)		0.06 (-0.09-0.21	.45	0%
Overall	4		748	11.1 (10.8-11.5)		757	11.2 (11.0-11.3)		0.07 (-0.08-0.22	.35	0%
Maternal anaemia (<11g/dL)											
HIV-positive	2	42	90	540 (443-636)	38	89	475 (313-729)	0.88 (0.58-1.35)	65 (-189-227)	.57	18%
HIV-negative	3	284	658	429 (317-469)	286	668	425 (373-480)	0.99 (0.87-1.12)	4 (-51-56)	.85	0%
Overall	4	326	748	443 (317-636)	324	757	434 (385-487)	0.98 (0.87-1.10)	9 (-44-58)	.72	0%
Moderate-severe maternal anaemia (<8 or <=7 or <=6 g/dL)											
HIV-positive	1	0	11	00 (00-00)	1	11	00 (00-00)	3.00 (0.14-66.53	0 (0-0)	.49	-
HIV-negative	3	13	658	19 (09-63)	15	668	21 (10-46)	1.12 (0.52-2.40)	-2 (-27-9)	.77	0%
Overall	3	13	669	14 (00-63)	16	679	17 (08-35)	1.18 (0.56-2.48)	-3 (-21-6)	.66	0%
Maternal parasitemia											
HIV-positive	2	1	88	06 (00-13)	1	94	06 (00-89)	0.94 (0.06-14.75	0 (-83-6)	.96	-[d]
HIV-negative	3	99	676	92 (31-263)	96	675	89 (69-114)	0.97 (0.75-1.24)	3 (-22-23)	.79	0%
Overall	4	100	764	31 (00-263)	97	769	30 (23-38)	0.97 (0.75-1.24)	1 (-7-8)	.79	0%
Placental malaria											
HIV-positive	2	1	85	07 (00-14)	2	91	13 (01-142)	1.87 (0.17-20.23	-6 (-135-6)	.61	-[d]
HIV-negative	2	32	290	71 (00-143)	17	290	48 (11-210)	0.68 (0.16-2.96)	23 (-139-60)	.61	31%
Overall	3	33	375	07 (00-143)	19	381	05 (02-14)	0.71 (0.26-1.95)	2 (-7-5)	.50	21%

Table 4. 4 Continued

	No. of studies	No. Events	Total No.	ACR[a] per 1000 or ACM[b] (Range)	No. Events	Total No.	CIR[a] per 1000 or CIM[b] (95% CI)	Relative Risk (95% CI)[c]	ARR per 1000 or Mean Difference (95% CI)[c]	P-Value[c]	I^2
Preterm delivery											
HIV-positive	2	47	116	378 (306-450)	45	124	242 (64-896)	0.64 (0.17-2.37)	136 (-518-314)	.51	82%
HIV-negative	3	85	776	94 (77-137)	83	804	82 (46-145)	0.87 (0.49-1.54)	12 (-51-48)	.63	66%
Overall	3	132	892	137 (77-450)	128	928	116 (77-174)	0.85 (0.56-1.27)	21 (-37-60)	.42	65%
Miscarriage											
HIV-positive	1	0	36	00 (00-00)	0	43	(-)	. (.-.)	. (.-.)		-
HIV-negative	3	10	792	00 (00-27)	9	812	00 (00-00)	0.90 (0.37-2.19)	0 (0-0)	.82	-[d]
Overall	3	10	828	00 (00-27)	9	855	00 (00-00)	0.90 (0.37-2.19)	0 (0-0)	.82	-[d]
Stillbirth											
HIV-positive	1	2	36	56 (56-56)	0	43	10 (01-190)	0.17 (0.01-3.39)	46 (-134-55)	.24	-
HIV-negative	3	20	792	30 (16-36)	29	812	42 (22-78)	1.39 (0.74-2.59)	-12 (-48-8)	.30	15%
Overall	3	22	828	33 (16-56)	29	855	41 (20-82)	1.24 (0.61-2.50)	-8 (-49-13)	.55	29%
Neonatal death[e]											
HIV-positive	1	1	34	29 (29-29)	2	43	46 (04-485)	1.58 (0.15-16.71	-17 (-456-25)	.70	-
HIV-negative	3	10	771	15 (08-19)	14	802	18 (07-51)	1.23 (0.44-3.38)	-3 (-36-8)	.69	27%
Overall	3	11	805	17 (08-29)	16	845	22 (10-50)	1.31 (0.59-2.93)	-5 (-33-7)	.51	0%

Abbreviation: No, number; 3+dose, monthly or 3 doses of sulfadoxine-pyrimethamine; ACR, Assumed Control-group Risk; ACM, Assumed Control-group Median; CIR Corresponding Intervention-group Risk; CIM, Corresponding Intervention-group Median; ARR, Absolute Risk Reduction (risk difference), HIV, Human Immunodeficiency Virus; RR, summary Relative risk adjusted by stratification for gravidity group (G1-G2, G3+) obtained from randomeffect models ; CI, Confidence Interval; g/dl, gram per deciliter; I^2, statistical test for heterogeneity across trials.

[a] Assumed Control-Group Risks represents the median risk (expressed per 1000 women) across the trials in the 2-dose arm; the Corresponding Intervention-Group Risk (and its 95% CI) is based on the assumed risk in 3+dose recipients computed as ACR x RR.(Schünemann et al., 2008) For the 2 continuous endpoints, the assumed Control-Group Median represents the median birthweight or haemoglobin concentration in the 2-dose arm (the range is provided to illustrate high and low risk populations); The corresponding Intervention-Group values were computed as the ACM + Mean Difference (95% CI).

[b] The absolute risk reduction (ARR) was calculated as the ACRx(1-RR) and expressed per 1000 women.

[c] Effect size, 95% confidence intervals and P-values for the overall effect (last rows) and for each HIV-status subgroup were obtained from random effects models and are adjusted for HIV status (for last rows representing the overall effect), by using the independent subgroups as the unit of analysis.

[d] Heterogeneity cannot be estimated, either because the data contains only a single trial in the subgroup, or no events occurred in 1 of the 2 included studies (Parise et al., 1998)

[e] Death of a live-born baby within the first 28 days of life. One study assessed early neonatal death only (death within 7 days of life).(MacArthur et al., 2007)

Chapter 4

Table 4. 5: Fixed effects meta-analysis of trials comparing the 2-dose vs 3+ doses of IPTp with SP by HIV status and gravidity groups

	All Gravidae			Primi, and secundigravidae			Multigravidae (G3+)		
	RR (95% CI) or Mean Difference (95% CI) [a]	P-Value	I^2	RR (95% CI) or Mean Difference (95% CI) [a]	P-Value	I^2	RR (95% CI) or Mean Difference (95% CI) [a]	P-Value	I^2
Primary Endpoints									
Low Birthweight									
HIV-positive	0.81 (0.58, 1.15)	.54	33%	0.84 (0.57, 1.23)	.37	0%	0.74 (0.35, 1.56)	.43	80%
HIV-negative	0.76 (0.62, 0.93)	.01	0%	0.75 (0.60, 0.95)	.02	7%	0.78 (0.52, 1.18)	.24	0%
Unknown	0.86 (0.62, 1.19)	.37	-[b]	0.86 (0.62, 1.19)	.37	-[b]	-	.16	30%
Overall	0.79 (0.68, 0.92)	.003	0%	0.80 (0.67, 0.94)	.009	0%	0.77 (0.54, 1.10)		
Birthweight, grams									
HIV-positive	97 (22, 172)	.01	0%	102(12, 192)	.03	0%	80 (-49, 221)	.21	53%
HIV-negative	58 (26, 90)	<.001	0%	67 (20, 114)	.005	0%	50 (6, 94)	.03	0%
Unknown	11 (-57, 79)	.75	-[b]	11.(-57, 79)	.75	-[b]	-	.01	0%
Overall	56 (29, 83)	<.001	0%	57 (22, 93)	.002	0%	53 (12, 95)		
Secondary Endpoints									
Maternal haemoglobin, g/dL									
HIV-positive	0.11 (-0.15, 0.37)	.40	0%	0.08 (-0.21-0.38)	.58	0%	0.20 (-0.33, 0.73)	.46	0%
HIV-negative	0.15 (0.04, 0.26)	.009	0%	0.24 (0.08-0.39)	.003	0%	0.06 (-0.09, 0.21)	.45	0%
Unknown	0.00 (-0.31, 0.31)	1	-[b]	0.00 (-0.31-0.31)	1	-[b]	-	.35	0%
Overall	0.13 (0.03, 0.22)	.009	0%	0.17 (0.04-0.30)	.008	0%	0.07 (-0.08, 0.22)		
Maternal anaemia (<11g/dL)									
HIV-positive	0.93 (0.82, 1.05)	.23	0%	0.94 (0.83, 1.06)	.33	0%	0.91 (0.66, 1.27)	.59	18%
HIV-negative	0.97 (0.89, 1.04)	.37	0%	0.95 (0.86, 1.04)	.28	0%	0.99 (0.87, 1.12)	.86	0%
Unknown	0.88 (0.75, 1.03)	.59	-[b]	0.88 (0.75, 1.03)	.11	-[b]	-	.73	0%
Overall	0.94 (0.89, 1.00)	.06	0%	0.93 (0.87, 1.00)	.042	0%	0.98 (0.87, 1.10)		
Moderate-severe maternal anaemia (<8 or <=7 or <=6 g/dL)									
HIV-positive	0.44 (0.13, 1.50)	.19	48%	0.26 (0.06, 1.23)	.09	-[c]	3.00 (0.14, 66.53)	.49	-[b]
HIV-negative	0.68 (0.42, 1.10)	.12	34%	0.44 (0.22, 0.87)	.02	37%	1.16 (0.56, 2.42)	.69	0%
Unknown	0.85 (0.48, 1.50)	.57	0%	0.85 (0.48, 1.50)	.57	0%	-	.57	0%
Overall	0.71 (0.50, 1.01)	.06	15%	0.59 (0.39, 0.89)	.01	20%	1.23 (0.60, 2.50)		
Maternal parasitemia									
HIV-positive	0.26 (0.15, 0.46)	<.001	0%	0.25 (0.14, 0.44)	<.001	0%	0.94 (0.06, 14.75)	.96	-[b]
HIV-negative	0.86 (0.74, 1.00)	.054	0%	0.80 (0.66, 0.97)	.02	5%	0.97 (0.75, 1.25)	.81	0%
Unknown	0.29 (0.06, 1.37)	.12	-[b]	0.29 (0.06, 1.37)	.12	-[b]	-	.80	0%
Overall	0.75 (0.65.0.87)	<.001	47%	0.66 (0.55. 0.79)	<.001	56%	0.97 (0.75, 1.23)		

Table 4.5 (continued)

	All Gravidae			Primi, and secundigravidae			Multigravidae (G3+)		
	RR (95% CI) or Mean Difference (95% CI) [a]	P-Value	I^2	RR (95% CI) or Mean Difference (95% CI) [a]	P-Value	I^2	RR (95% CI) or Mean Difference (95% CI) [a]	P-Value	I^2
Placental malaria									
HIV-positive	0.38 (0.22, 0.68)	.001	0%	0.34 (0.19, 0.63)	.0006	0%	1.87 (0.17, 20.23)	.61	- [b]
HIV-negative	0.56 (0.40, 0.78)	<.001	9%	0.58 (0.38, 0.89)	.01	23%	0.52 (0.30, 0.91)	.02	31%
Unknown	0.57 (0.17, 1.94)	.37	- [b]	0.57 (0.17, 1.94)	.37	- [b]	-	.03	21%
Overall	0.51 (0.38, 0.67)	<.001	0%	0.49 (0.35, 0.68)	<.001	0%	0.56 (0.33, 0.96)		
Preterm delivery									
HIV-positive	0.90 (0.77, 1.09)	.30	32%	0.91 (0.73, 1.12)	.35	0%	0.92 (0.68, 1.25)	.60	82%
HIV-negative	0.90 (0.75, 1.07)	.23	41%	0.86 (0.68, 1.08)	.19	26%	0.95 (0.72, 1.27)	.74	66%
Unknown	1.29 (0.91, 1.83))	.15	1%	1.29 (0.91, 1.83)	.15	1%	-	.58	65%
Overall	0.95 (0.84, 1.08)	.44	35%	0.96 (0.83, 1.11)	.58	7%	0.94 (0.76, 1.17)		
Miscarriage									
HIV-positive	1.54 (0.38, 6.28)	.55	- [b]	1.54 (0.38, 6.28)	.55	- [b]			
HIV-negative	1.38 (0.78, 2.43	.27	20%	1.84 (0.86, 3.92)	.11	19%	0.90 (0.37, 2.19)	.82	- [b]
Unknown	1.80 (0.61, 5.34)	.29	- [b]	1.80 (0.61, 5.34)	.29	- [b]	-	.82	- [b]
Overall	1.47 (0.92, 2.36)	.55	0%	1.78 (1.01, 3.14)	.046	0%	0.90 (0.37, 2.19)		
Stillbirth									
HIV-positive	0.68 (0.27, 1.68)	.40	0%	1.9 (0.36, 3.98)	.78	0%	0.17 (0.01, 3.39)	.24	- [b]
HIV-negative	1.34 (0.91, 1.96)	.14	0%	1.28 (0.76, 2.15)	.36	0%	1.40 (0.80, 2.46)	.23	15%
Unknown	1.00 (0.57, 1.75)	1	54%	1.00 (0.57, 1.75)	1.00	54%	-	.40	29%
Overall	1.14 (0.85, 1.53)	.38	0%	1.14 (0.80, 1.64)	.47	0%	1.26 (0.74, 2.16)		
Neonatal death [c]									
HIV-positive	0.48 (0.18, 1.32)	.16	0%	0.36 (0.11, 1.15)	.08	0%	1.58 (0.15, 16.71)	.70	- [b]
HIV-negative	1.18 (0.70, 1.99)	.53	0%	1.07 (0.54, 2.14)	.84	0%	1.34 (0.60, 3.00)	.47	27%
Unknown	0.50 (0.20, 1.22)	.13	37%	0.50 (0.20, 1.22)	.13	37%	-	.42	0%
Overall	0.84 (0.56, 1.26)	.40	0%	0.69 (0.43, 1.12)	.13	0%	1.37 (0.64, 2.93)		

Abbreviation: No, number; 3+dose, monthly or 3 doses of sulfadoxine-pyrimethamine; HIV, Human Immunodeficiency Virus; RR, summary Relative Risk; CI, Confidence Interval; g/dl, gram per deciliter; I^2, statistical test for heterogeneity across trials.

[a] Effect size, 95% confidence intervals and P-values for the overall effect (last rows) and for each HIV-status subgroup were obtained from fixed effects models. The overall effect estimates (last rows) are adjusted for HIV status by using the independent subgroups as the unit of analysis.

[b] Heterogeneity cannot be estimated, either because the data contains only a single trial in the subgroup, or no events occurred in 1 of the 2 included studies (Parise et al., 1998)

[c] Death of a live-born baby within the first 28 days of life. One study assessed early neonatal death only (death within 7 days of life).(MacArthur et al., 2007)

There was no evidence for publication bias after visual inspection of funnel plots or with the Harbord modified test for small-study effects (P = .74) (Figure 4. 5). Cumulative meta-analysis, ordered by publication date, showed that a significant association with LBW emerged with the addition of new evidence from trials reported since 2010 (Figure 4.6 & Figure 4.7). Sensitivity analysis showed that after removal of both low-quality studies, the point estimates for LBW and mean birth weight were RR = 0.76 (95% CI, 0.61-0.93), I^2 = 16%; and mean difference = 62 g (95% CI, 29-95 g), I^2 = 0%. Removal of any individual trial also had relatively little effect and pooled results remained statistically significant at P < .05 for all 7 analyses with fixed-effects models and at P = .06 with random-effects models (Figure 4. 8 & Figure 4.9).

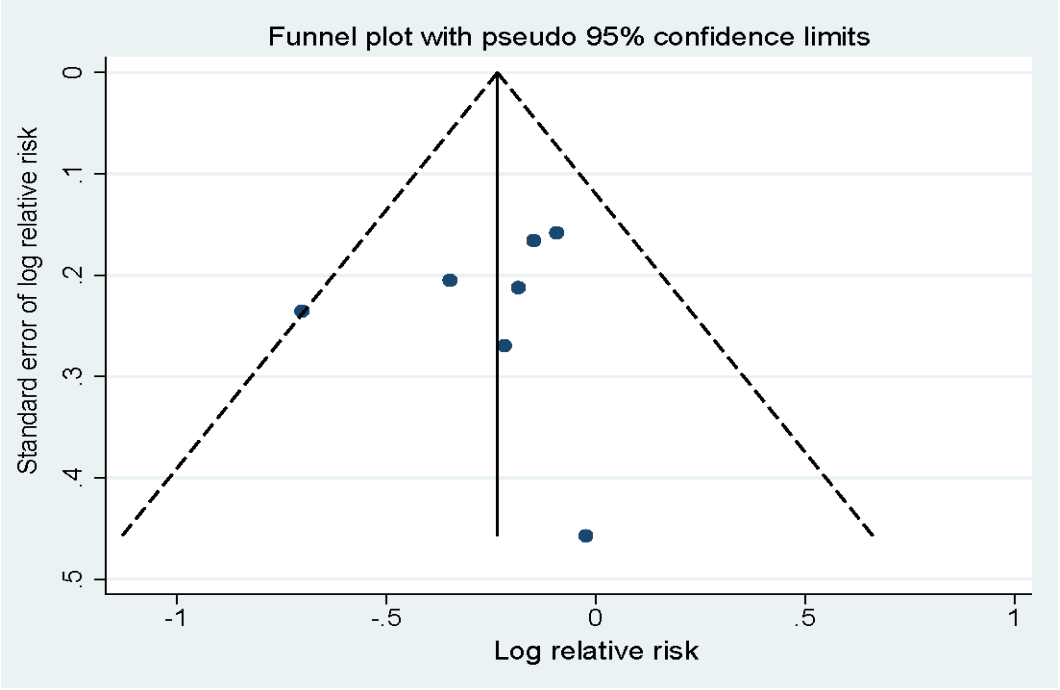


Figure 4. 5: Funnel plot of standard error by log relative risk
Notes: Dots represent each study's log Relative Risk for the effect on low birthweight. Harbord's modified test for small-study effects: P=.74

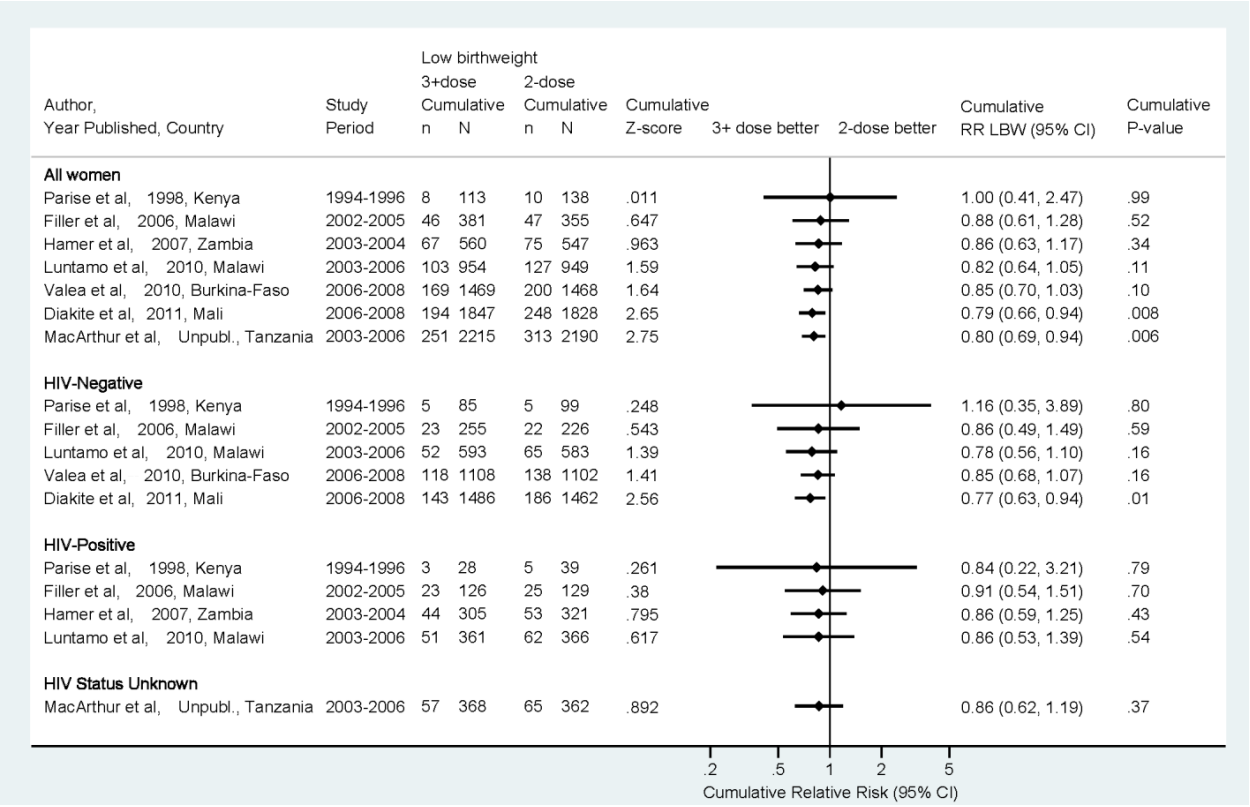


Figure 4.6: Cumulative Meta-analysis of the association with low birthweight, ranked by year of publication, overall and by HIV status

Figure note: n, cumulative number of events; N, cumulative total number of women per treatment arm; LBW, low birthweight (<2500 gram), RR, relative risk; CI, confidence interval; HIV, human immune deficiency virus. Dots represent composite estimates for the effect on low birthweight adjusted for HIV status (upper strata 'All women') and gravidity (all 3 strata) obtained by performing random effect meta-analysis (Mantel Haenszel) on the HIV and gravidity subgroups for each study. Reference numbers refer to those in the main manuscript.

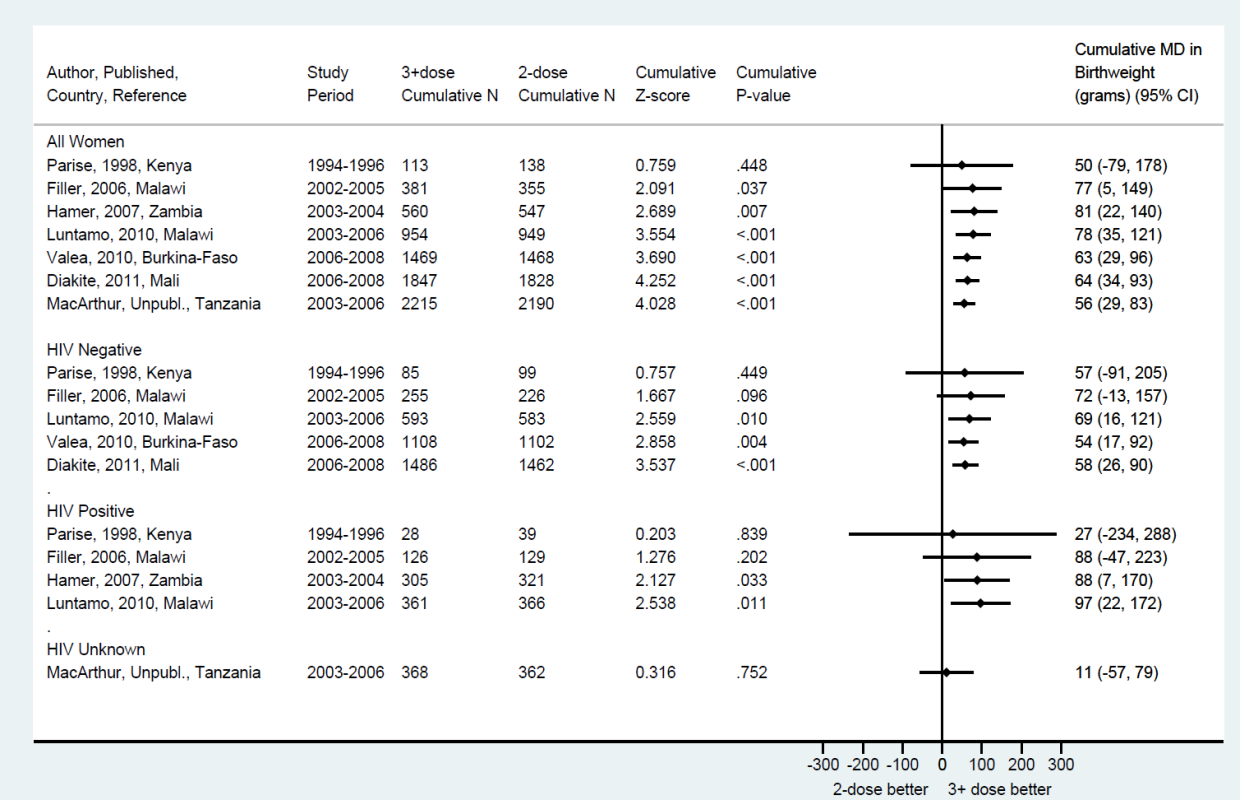


Figure 4.7: Cumulative Meta-analysis of the association with mean birthweight, ranked by year of publication, overall and by HIV status

Figure note: N, Cumulative number of women per treatment arm. CI, confidence interval; HIV, human immune deficiency virus. Dots represent composite estimates for the effect on mean birthweight adjusted for HIV status (upper strata 'All women') and gravidity (all 3 strata) obtained by performing random effect meta-analysis (inverse variance) on the HIV and gravidity subgroups for each study. Reference numbers refer to those in the main manuscript.

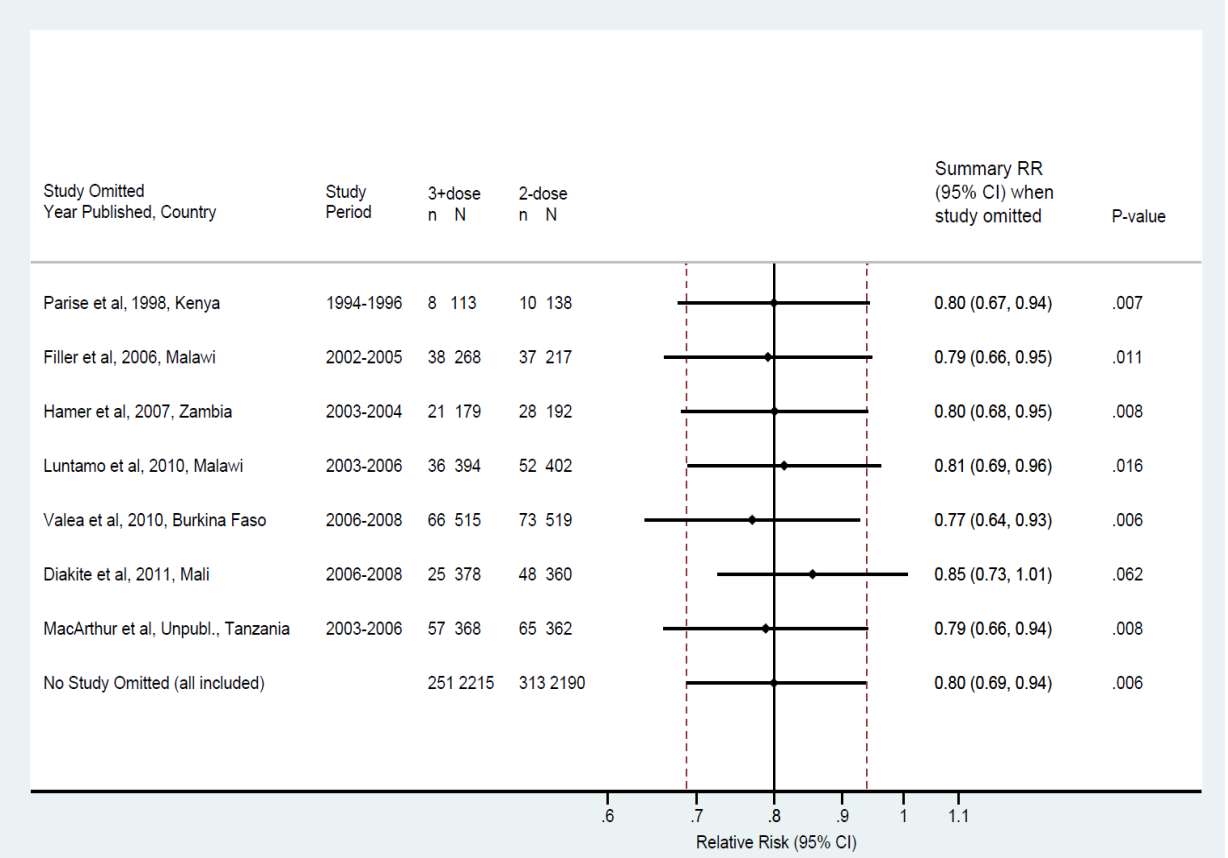


Figure 4.8: Influence of removing 1 study at a time on the meta-analysis summary estimates for low birthweight

Figure note: CI, Confidence Interval. RR, Relative Risk; LBW, low birthweight. The number of events (n) and total number (N) are the crude sums of all events and women within each The summary RRs for low birthweight are the weighted average of the trial-specific relative risks. The trial specific relative risks represent the composite point estimates for each study obtained by performing random effects meta-analysis on the HIV and gravidity subgroups for that study. The solid vertical line represents the pooled effect estimate with all studies included and the 2 broken vertical lines in red represent the corresponding 95% CI. When fixed effects models were used (not shown), the pooled results remained statistically significant at P<0.05 for all 7 analyses, also for the study by Diakite et al, 2011, from Mali (RR=0.85, 0.77-0.996). Reference numbers refer to those in the main manuscript.

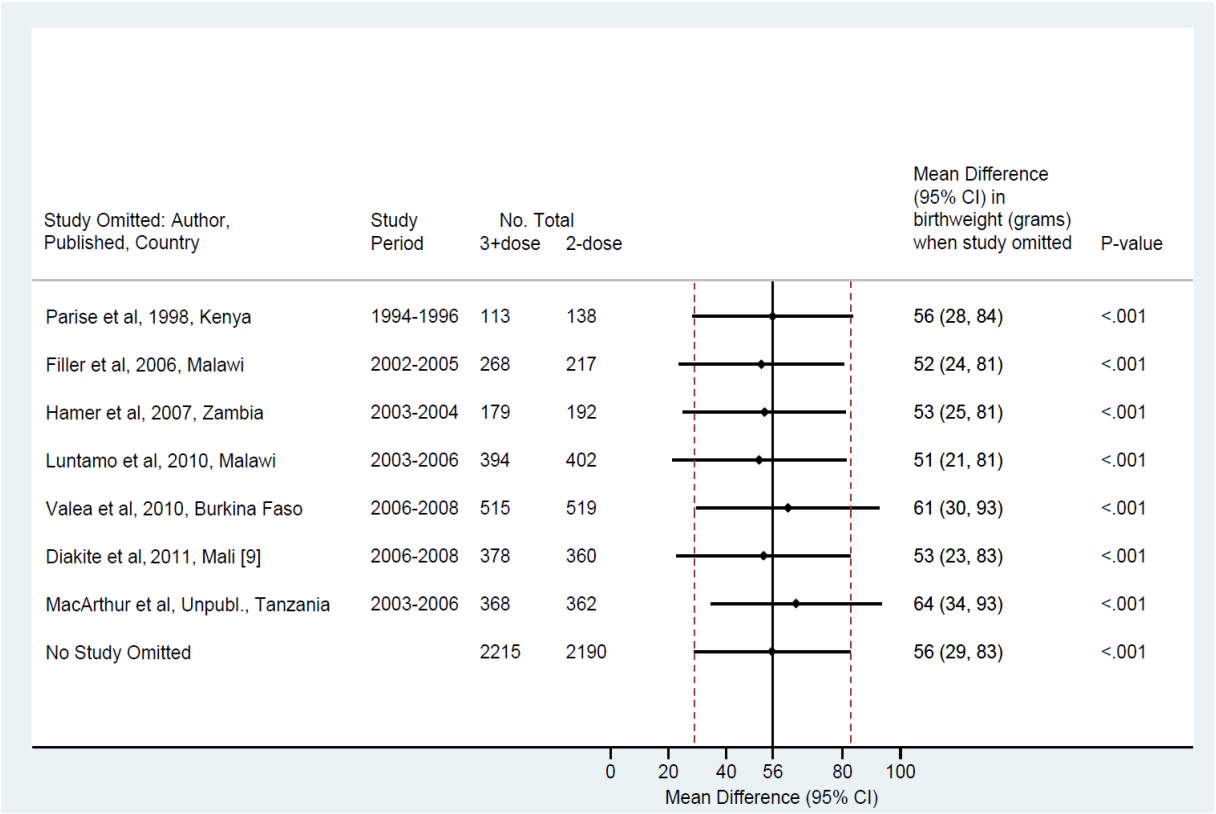


Figure 4.9: Influence of removing 1 study at a time on the meta-analysis summary estimates for differences in mean birthweight

Figure note: CI, Confidence Interval. No., Cumulative number of women. The summary estimates for the mean differences are the weighted averages of the trial-specific mean differences. The trial specific mean differences represent the composite point estimate for each study obtained by performing random effects meta-analysis on the HIV and gravidity subgroups for that study. The solid vertical line represents the pooled effect estimate with all studies included and the 2 broken vertical lines in red represent the corresponding 95% CI. Reference numbers refer to those in the main manuscript.

4.3.3 Secondary outcomes

The median maternal haemoglobin level at term in the 2-dose group was 10.9 g/dL (range, 9.7-11.6 g/dL), and this was on average 0.13 g/dL higher (95% CI, 0.03-0.22 g/dL) in the ≥3-dose group (Figure 4.10 & Table 4. 2).

This group had a lower risk of moderate to severe maternal anaemia, but this was evident only in G1-G2 women (RR = 0.60 [95% CI, 0.36-0.99]; I^2 = 20%), not overall (RR = 0.73 [95% CI, 0.48-1.11]; I^2 = 15%) (Figure 4.11 & Table 4. 2). Women in the ≥3-dose group were approximately half as likely to have placental malaria (6 studies) compared with those in the 2-dose group, regardless of HIV status (RR = 0.51 [95% CI, 0.38-0.68]; I^2 = 0%) (Table 4. 2 & Figure 4.12), but this was evident only in G1-G2 women (RR = 0.50 [95% CI, 0.35-0.70]; I^2 = 0%) (Table 4. 3), not in multigravida (RR = 0.71 [95% CI, 0.26-1.95]; I^2 = 21%) (Table 4.4) . Similarly, ≥3 doses were associated with less peripheral (maternal) malaria (RR = 0.68 [95% CI, 0.52-0.89]; I^2 = 47%) (Table 4. 2), but this was evident in G1-G2 women only (RR = 0.54 [95% CI, 0.37-0.80]; I^2 = 56%) (Table 4. 3), not in multigravida (RR = 0.97 [95% CI, 0.75-1.24]; I^2 = 0%) (Table 4. 4). No difference in preterm delivery was detected (RR = 0.95 [95% CI, 0.80-1.12]; I^2 = 35%) or in the number of stillbirths (RR = 1.14 [95% CI, 0.85-1.55]; I^2 = 0%), miscarriages (RR = 1.43 [95% CI, 0.88-2.33]; I^2 = 0%), or neonatal deaths (RR = 0.88 [95% CI, 0.57-1.35]; I^2 = 0%) (Table 4. 2).

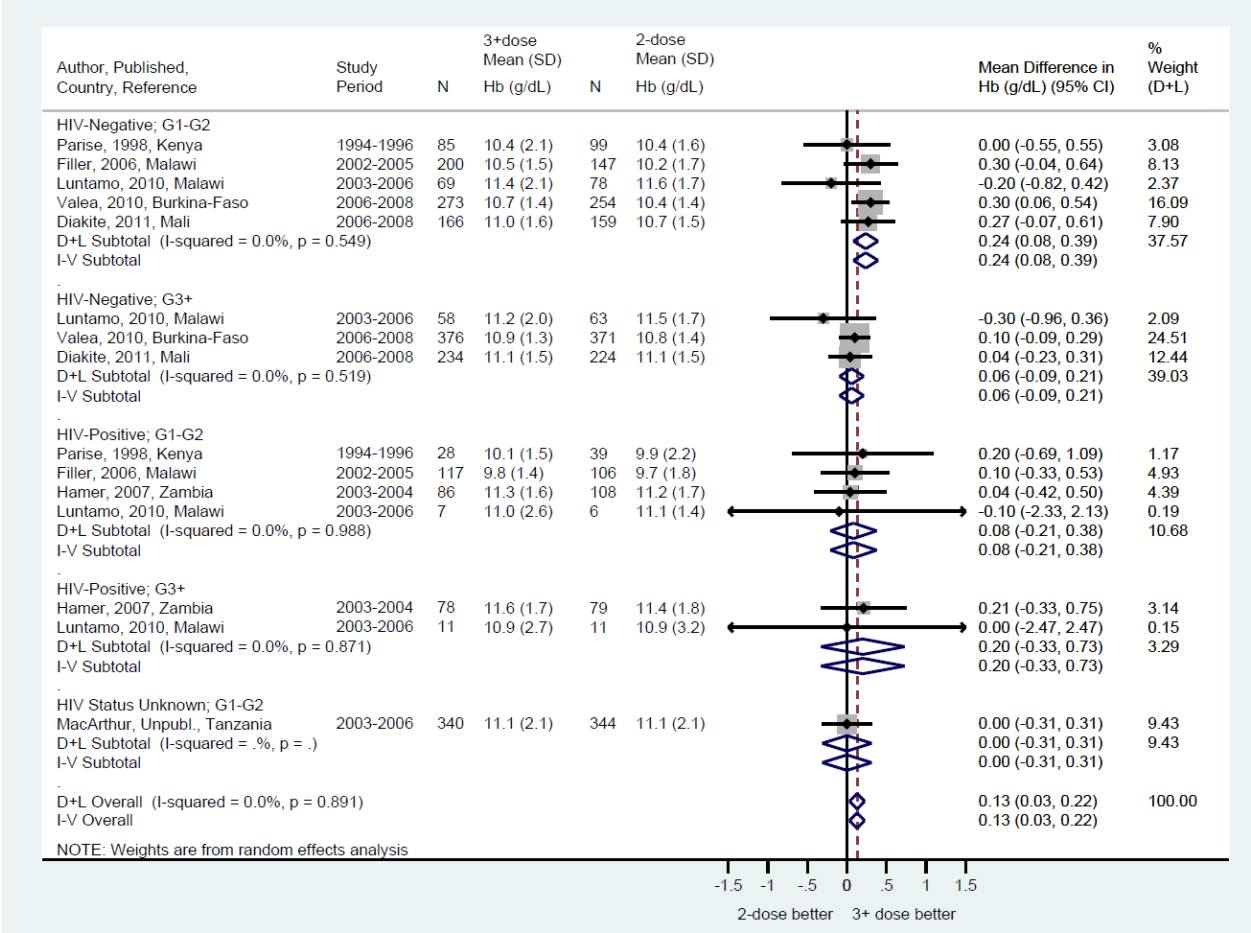


Figure 4.10: Meta-analysis of mean maternal haemoglobin at birth in trials comparing the 2-dose vs 3+ doses of IPTp with sulfadoxine-pyrimethamine

Figure note: N, sample size; HIV, human immunodeficiency virus status; G1-G2, first and second pregnancies; G3+, 2 or more previous pregnancies; CI, confidence interval; D+L, Dersimonian-Laird method for random effects models; I-V, Inverse-Variance method used in the fixed effects models; SD, Standard Deviation. Data-marker sizes indicate the weight applied to each study using random-effects meta-analysis. Diamonds represent summary effect of studies. P-values following the I^2 statistics represent the Chi-square test for heterogeneity. Reference numbers refer to those in the main manuscript.

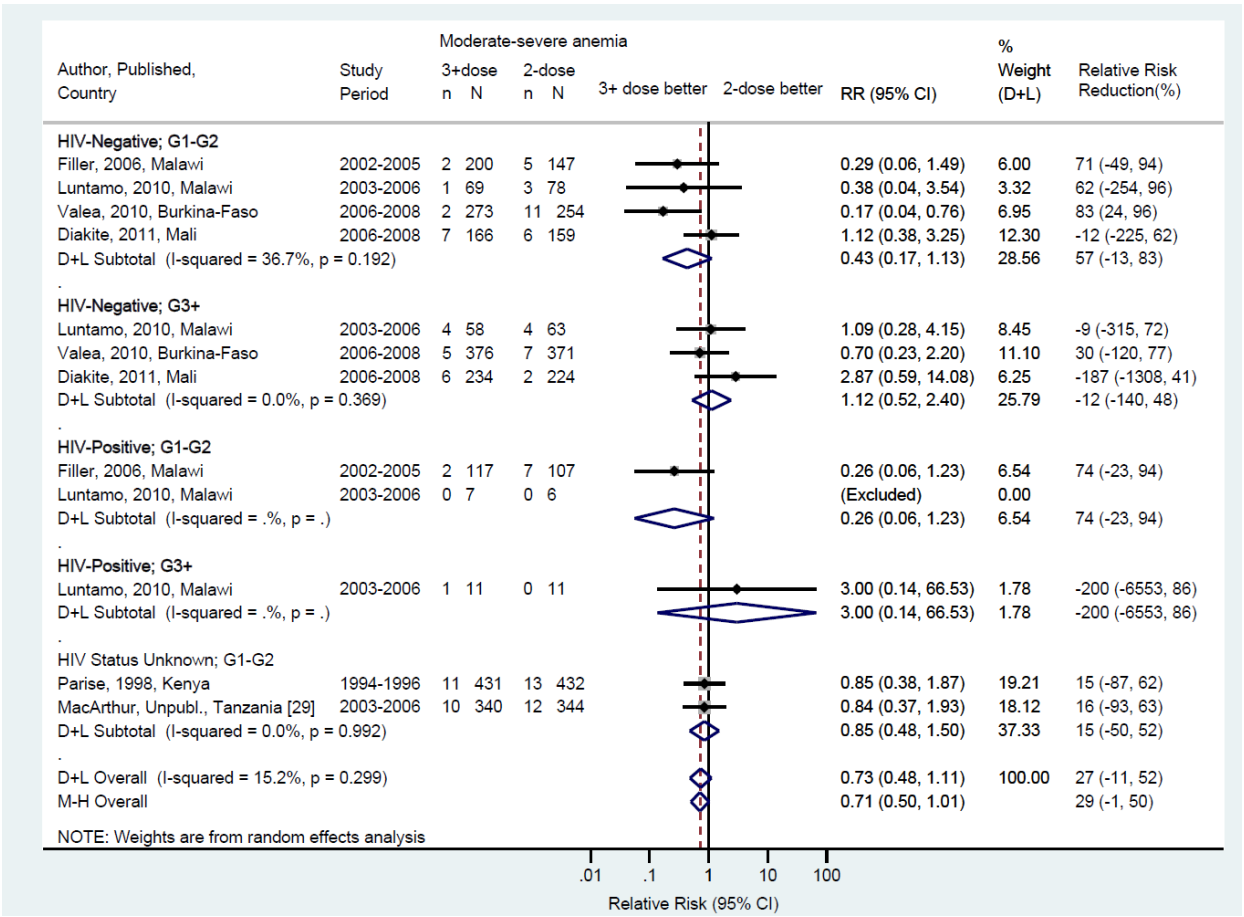


Figure 4.11:Meta-analysis of moderate-severe maternal anaemia at birth in trials comparing the 2-dose vs 3+ doses of IPTp with sulfadoxine-pyrimethamine

Figure note: n, number of events; N, sample size; HIV, human immunodeficiency virus status; G1-G2, first and second pregnancies; G3+, 2 or more previous pregnancies; CI, confidence interval. D+L, Dersimonian-Laird method for random effects models; M-H, Mantel-Haenszel method for fixed effects models. Moderate-severe maternal anaemia was defined as haemoglobin concentrations <8 or <=7 or <=6 g/dL, depending on the trial definition used. Data marker sizes indicate the weight applied to each study using random-effects meta-analysis. Diamonds represent summary effect of studies. Moderate-severe anaemia was defined by the individual trials as Hb <6, 7 or 8 g/dl. P-values following the I^2 statistics represent the Chi-square test for heterogeneity. Reference numbers refer to those in the main manuscript.

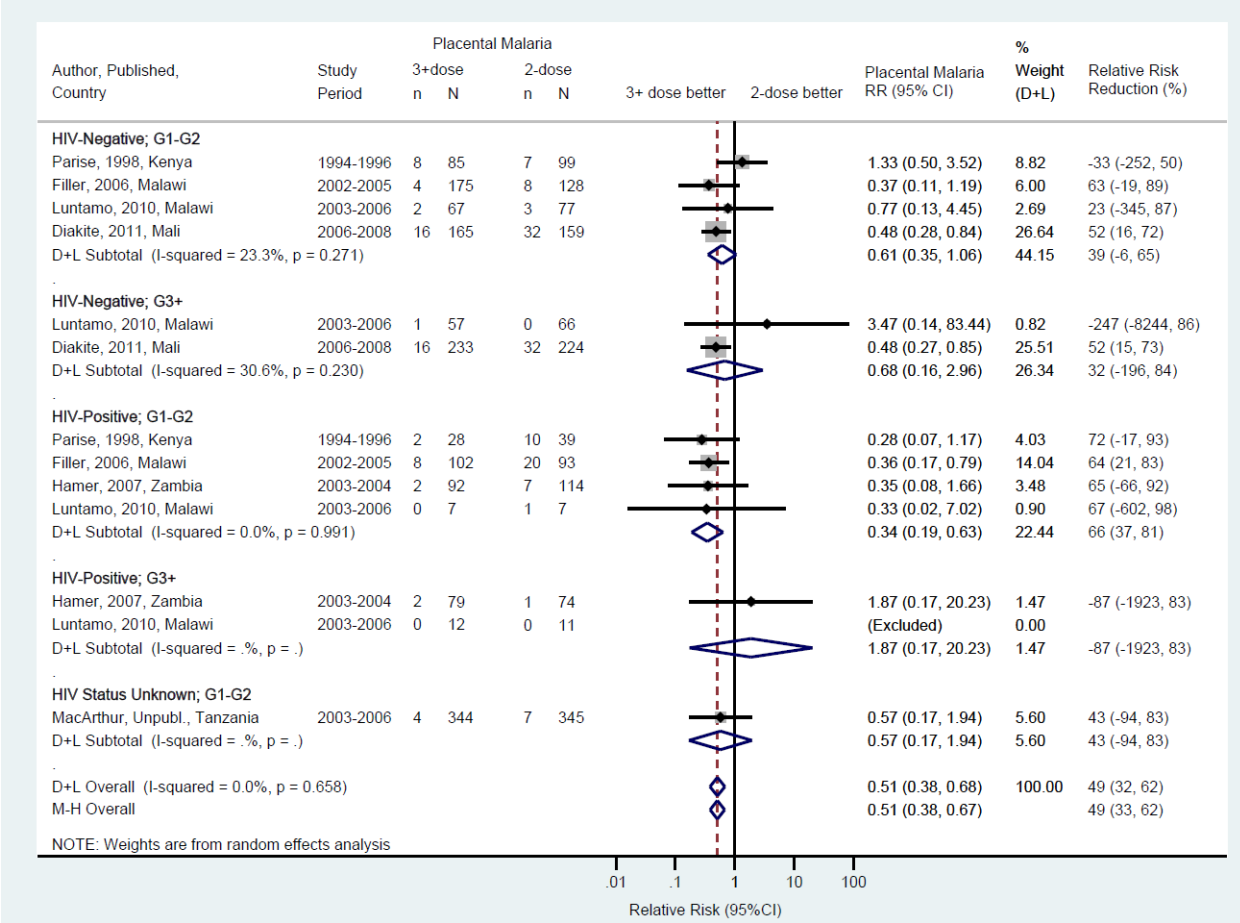


Figure 4.12: Meta-analysis of placental malaria in trials comparing the 2-dose vs 3+ doses of IPTp with sulfadoxine-pyrimethamine

Figure note: n, number of events; N, sample size; HIV, human immunodeficiency virus status; G1-G2, first and second pregnancies; G3+, 2 or more previous pregnancies; CI, confidence interval. D+L, Dersimonian-Laird method for random effects models; M-H, Mantel-Haenszel method for fixed effects models. Data-marker sizes indicate the weight applied to each study using random-effects meta-analysis. Diamonds represent summary effect of studies. P-values following the I^2 statistics represent the Chi-square test for heterogeneity. Reference numbers refer to those in the main manuscript.

4.3.4 Stratified analysis for low birth weight and mean birth weight

There was no clear correlation between resistance level and the strength of the association between treatment regimen and LBW or mean birth weight; the point estimates were similar in areas with less than 50% *DHPS* -K540E mutations (5 trials) and areas with 50% or more *DHPS* -K540E (2 trials) (Figure 4.13 & Figure 4.14). There was also no evidence that intensity of malaria transmission or the median number of sulfadoxine-pyrimethamine doses in the ≥3-dose group modified the association (*P* > .17 for all tests for subgroup differences). There was no clear difference in the association between the dose group and the risk of LBW or mean birth weight in the 2 trials that used high-dose folate supplementation (5 mg/d) (Hamer et al., 2007, Parise et al., 1998) (which has since been contraindicated) vs the standard dose (0.25-0.5 mg/d). Three studies reported results stratified by insecticide-treated net use (Diakite et al., 2011, Luntamo et al., 2010, MaArthur, 2005); the associations with LBW and mean birth weight were statistically significant in the nonusers only. There was no evidence for an association with LBW in insecticide-treated net users (Figure 4.13 & Figure 4.14).

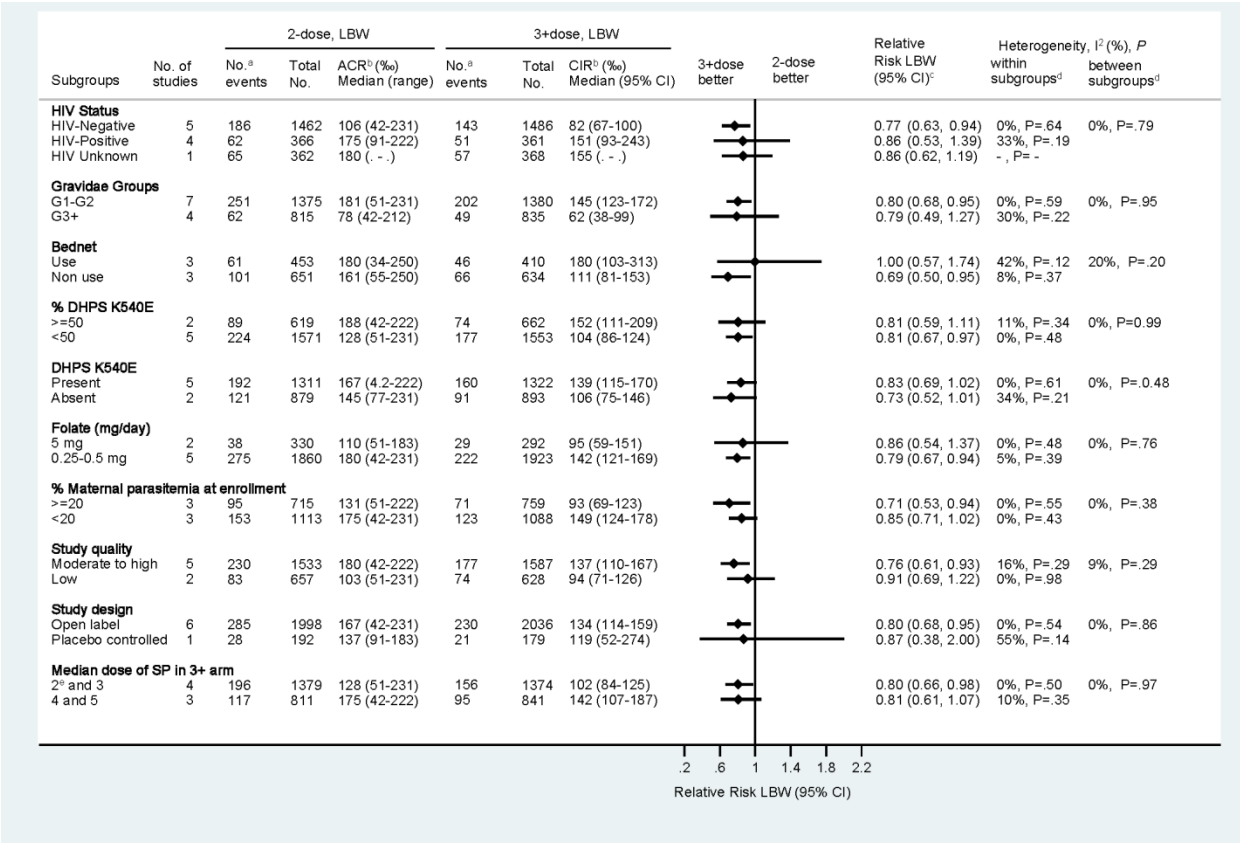


Figure 4.13:: Subgroup analysis and analysis of determinants of the association with low birthweight

Figure notes: n, number of events; N, total number; G1-G2, first and second pregnancy; G3+, 2 or more previous pregnancies; DHPS-540E, dihydropteroate synthase 540 mutation; LBW, Low birthweight defined as a birthweight <2500 grams. ACR, Assumed Control-group Risk; CIR, Assumed Intervention-group Risk; HIV, human immunodeficiency virus status; mg, milligram; CI, confidence interval; SP, sulfadoxine-pyrimethamine

[a] number events (n) and total number (N) are the crude sums of all events and women across studies within each subgroup.

[b] The ACR represents the observed median risk (expressed per 1000 women) and range for each subgroup in the 2-dose arm. The CIR is the corresponding risk in the 3-dose group computed as the ACR x RR (95% CI).

[c] The relative risks are the weighted averages of the trial or subgroup-specific relative risks within each strata (row), adjusted for HIV status and gravidity obtained from random effects models, expect for the analysis of HIV status itself (first 4 rows), which was adjusted for gravidity only, and the analysis of gravidity (rows 5 to 7), which was adjusted for HIV status only

[d] Heterogeneity relating to the extent that the RRs vary between trials or subgroups are shown as the I^2 statistic, which depicts the % of the between-study or between subgroup heterogeneity that is attributable to variability in the effect, rather than sampling variation. The corresponding P-value is based on the Chi-square statistic

[e] In one trial in Burkina Faso, the median number of doses in the 3+dose arm was only 2 because 2/3 of participants failed to receive the intended regimen. The median number of doses in the 2-dose group in that same trial was 1.

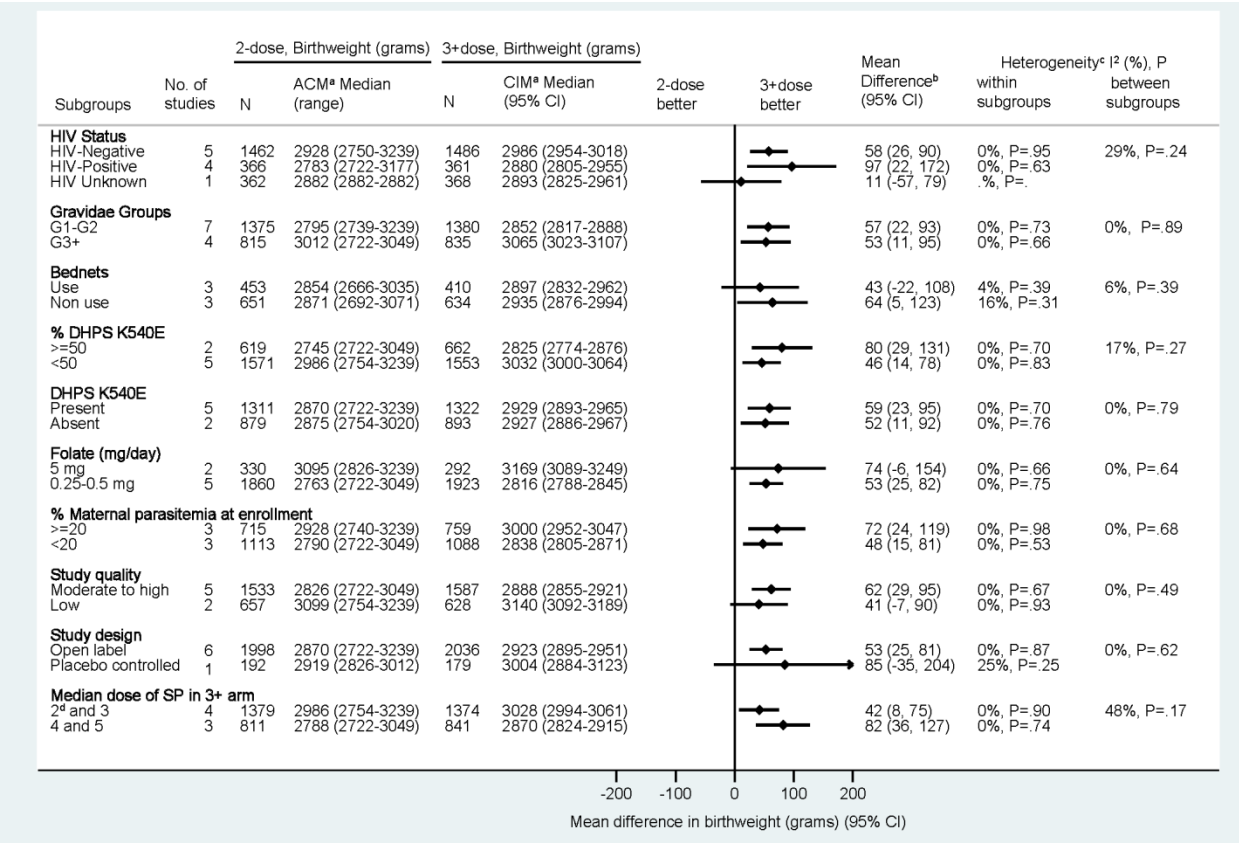


Figure 4.14: Subgroup analysis and analysis of determinants of the association with mean birthweight.

Figure notes: N, total number; G1-G2, first and second pregnancy; G3+, 2 or more previous pregnancies; DHPS-K540E, dihydropteroate synthase 540. ACM, Assumed Control-group Median; CIM, Corresponding Intervention-group Median; HIV, human immunodeficiency virus status; mg, milligram; CI, confidence interval; SP, sulfadoxine-pyrimethamine [a] The ACM represents the observed median and range for each subgroup in the 2-dose arm, CIM is the corresponding median in the 3-dose group computed as the ACM + the mean difference.

[b] The mean differences are the weighted averages of the trial or subgroup-specific mean differences within each strata (row), adjusted for HIV status and gravidity obtained from random effects models, expect for the analysis of HIV status itself (first 4 rows), which was adjusted for gravidity only, and the analysis of gravidity (rows 5 to 7), which was adjusted for HIV status only

[c] Heterogeneity relating to the extent that the RRs vary between trials or subgroups are shown as the I^2 statistic, which depicts the % of the between-study or between subgroup heterogeneity that is attributable to variability in the effect, rather than sampling variation. The corresponding P-value is based on the Chi-square statistic

[d] In one trial in Burkina Faso, the median number of doses in the 3+dose arm was only 2 because 2/3 of participants failed to receive the intended regimen. The median number of doses in the 2-dose group in that same trial was 1.

4.3.5 Adverse events

The risks of neonatal icterus and congenital malformation were comparable between the groups, as were the number of adverse events in the mother. One study reported a case of Stevens-Johnson syndrome, which occurred in the 3 or more dose group, 3 weeks after the first dose (Table 4. 6) (Hamer et al., 2007).

Table 4. 6:Summary of Adverse Events in Women and Neonates Following IPTp with 3+ versus 2-dose of sulfadoxine-pyrimethamine during pregnancy

	Sulfadoxine-pyrimethamine treatment		Neonatal Icterus, n/N(%) of newborns		Congenital abnormalities, n/N (%) of newborns		Maternal Drug Reaction to SP, n/N (%) of women		Severe Skin Reactions
Studies	Number of SP courses	Number of women	3+ dose	2-dose	3+ dose	2-dose	3+ dose	2-dose	
Parise et al. 1998	2276	1086[a]	60/431(14)	69/432(15)	Not reported		7/661(1.4)	14/680 (2.3)	None observed [b]
Filler et al. 2006	1734	641[a]	0.4%[c]		Not reported		<1%[c]		None observed
Hamer et al. 2007	1039	456	1/189 (0.5)	0/198 (0)	Not reported		1.13 (0.56-2.18)[d]		1 case reported in the monthly arm[e]
Luntamo et al. 2010	2603	877	Not reported		3/443 (0.7)	4/439(0.9)	Not reported		Not reported
Valea et al. 2010	2213	1296	Not reported		Not reported		Not reported		Not reported
Diakite et al. 2011	1997	814	11/400 (2.7)	10/383(2.5)	1/400 (0.3)	3/383(0.8)	0/413(0)	0/401(0)	None observed
McArthur et al	1692	799	14/272 (5.1)	21/290 (7.2)	5/383(1.3)	7/384(1.8)	23/399(5.7)[f]	28/400 (6.7)[f]	None observed
relative risk and 95% CI; I^2 (95% CI), P-value for heterogeneity			0.87 (0.66, 1.14); I^2=0%(0-61%), P=.76		0.65 (0.28, 1.50); I^2=0%(0-53), P=.80		0.73 (0.46, 1.15); I^2=0%(0-0), P=.38		

Notes: n, numerator representing the number of events; N, denominator representing the number assessed for the event; (%), percentage of the event unless otherwise indicated; 3+, monthly or 3 doses; IPT Intermittent Preventive Treatment; None observed, cases were assessed during the study but not observed by investigators; Not reported, cases were not reported in the article by authors; CI, confidence interval; I^2, I-squared, representing the percentage of heterogeneity between studies that contribute to the overall effect represented by the relative risk; P-value is the value of the probability testing for between studies heterogeneity

[a] Only reported for women followed prospectively

[b] in 193 treatment episodes among 94 HIV-positive and 502 treatment episodes among 230 HIV-negative women. Although no severe cutaneous reactions were observed, "two (2%) of 94 HIV-positive and none of 230 HIV-negative women had sulfadoxine-pyrimethamine withheld because of ADRs (mild rash or oral lesions)."

[c] Reported only for all groups pooled, but no statistical difference was observed between treatment groups.

[d] Numerator and denominators were not reported.

[e] The case of Stephen Johnson reported in the monthly arm occurred 3 weeks after the 1st dose of sulfadoxine-pyrimethamine.

[f] Maternal drug reactions collected from the 1st dose (enrolment) to the last dose including diarrhoea, rash, weakness, seizures, sleepiness, and difficulties to walk.

4.4 Comments and conclusion

This meta-analysis of 7 trials demonstrated that regimens of intermittent preventive therapy during pregnancy consisting of ≥3 doses of sulfadoxine-pyrimethamine were well tolerated and, compared with the 2-dose regimen, were associated with higher mean birth weight, less LBW, and less placental and maternal malaria at delivery. The ≥3-dose regimen was also associated with slightly higher mean maternal haemoglobin levels at term overall, but a significant association with moderate to severe maternal anaemia was observed only in G1-G2 women. The associations with birth weight were consistent across trials despite variations in study design, malaria endemicity, and the degree of sulfadoxine-pyrimethamine resistance. Although the number of trials was limited, there was no suggestion of publication or other small-study bias. There was also no suggestion that the results were affected by the weight of a single influential study. Two of the trials were classified as low quality, but sensitivity analysis indicated that their effect on the overall pooled estimate for LBW was minor. The consistency of these findings across the trials suggests the results are generalizable.

Although the summary point estimates of the association with mean birth weight were modest (56-g difference overall and 67 g among HIV-negative G1-G2 women), these were associated with clinically relevant changes in the risk of LBW, particularly among HIV-negative G1-G2 women (RR reduction = 25%). These estimates were comparable to that reported in previous studies for 2-dose intermittent preventive therapy during pregnancy relative to none (mean difference = 79 g; RR reduction = 29%) and for insecticide-treated nets alone (mean difference = 55 g; RR reduction = 23%) (Gamble et al., 2007, Ter Kuile and Steketee, 2007). The magnitude of the observed association is remarkable, given that approximately 28% of women were protected by insecticide-treated nets in these 7 trials and considering that the control group benefited from protection of the 2-dose intermittent preventive therapy during pregnancy with sulfadoxine-pyrimethamine. The association mainly reflects an association with foetal growth, rather than with preterm delivery, and indicates that more complete protection in the second and third trimesters, including the last 6 to 10 weeks of pregnancy, may be pivotal for foetal growth. This result is consistent with observations in healthy pregnancies, which show that of the total foetal weight gain,

28% and 55% of it occurs during the last 6 and 10 weeks of pregnancy, respectively (Mikolajczyk et al., 2011).

Although the lack of heterogeneity across the sulfadoxine-pyrimethamine resistance range is encouraging, it does not imply that sulfadoxine-pyrimethamine efficacy is unaffected at higher levels of resistance. A possible explanation is that the extra doses compensate for any reductions in efficacy of the 2-dose regimen resulting from a progressive decrease of the duration of post-treatment prophylaxis.

The association with placental infections is an expected outcome because the 3 or more dose group received their last dose on average 1 month closer to delivery and is likely to reflect clearance of existing infections near term and prevention of new infections by the extra period of prophylaxis. However, the association with mean birth weight among multigravida was unexpected because most multigravida in endemic countries have acquired a pregnancy-specific protective immunity during exposures in previous pregnancies. Overall, the evidence for a beneficial association in multigravida was weak, and the finding in this study may therefore reflect a chance observation (eg, because of multiple comparisons) or mechanisms other than the prevention of malaria. Although the point estimates for LBW (RR reduction 21%) and placental malaria (RR reduction 29%) were in the same direction as those observed in primigravida and secundigravida, none were statistically significant and there was no suggestion that ≥3 doses were associated with less maternal malaria or moderate to severe anaemia. On the other hand, the lack of significant association with LBW may reflect lack of power because only 4 of the 7 studies included multigravida.

Our meta-analysis has some limitations. First, although all trials were designed to standardize the number of visits and antenatal care (eg, hematinic supplementation) between the 2 groups, in one trial in Tanzania the women in the ≥3-dose group had on average 1 extra visit compared with the 2-dose group and thus potentially better antenatal care (MacArthur and Abdulla, 2005). However, exclusion of this study in the sensitivity analysis did not change the conclusion (Figure 4.8 & Figure 4.9). Second, only 1 of the 7 trials was placebo controlled, which may have biased the results and affected some outcomes because of lack of expectations in a 2-dose group or differential behaviors across intervention groups. We did not use blinding in the selection, evaluation, and data

abstraction phases, and because the authors were familiar with all included studies, this could have introduced bias (Jadad et al., 1996). Third, none of the trials were conducted in regions where additional *DHFR* 164L or *DHPS* 581G mutations are prevalent, as reported from parts of Rwanda, Uganda, and northern Tanzania, conferring the highest level of sulfadoxine-pyrimethamine resistance (Naidoo and Roper, 2011, Gesase et al., 2009, Harrington et al., 2011, Harrington et al., 2009). Last, only 3 trials reported results stratified by insecticide-treated net use, limiting our evaluation of the potential modifying role of insecticide-treated nets. In this smaller subgroup of studies, significant associations with LBW and mean birth weight were observed among the nonusers of insecticide-treated nets only, consistent with results of previous evaluations of 2-dose intermittent preventive therapy during pregnancy against placebo (van Eijk et al., 2012, Leke and Taylor, 2011, Peters et al., 2007).

Only 1 serious cutaneous reaction was reported in the current meta-analysis involving 13,554 sulfadoxine-pyrimethamine treatments among 6,281 pregnancies, and this occurred in an HIV-positive woman 3 weeks after she received her first dose of sulfadoxine-pyrimethamine for intermittent preventive therapy during pregnancy (Hamer et al., 2007). We found no indication that more frequent dosing (ie, resulting in doses administered closer to delivery) was associated with increased risk of neonatal jaundice, the main safety signal of interest in neonates. Sulfonamides have the potential to displace unconjugated bilirubin from albumin, which could increase a newborn's risk of kernicterus if received near delivery. Our observations, combined with the evidence reviewed by Peters et al (Peters et al., 2007) from the experience with sulfonamides for rheumatic fever prophylaxis, urinary tract infections, and congenital toxoplasmosis (which involve higher doses and prolonged use of sulfadoxine-pyrimethamine), suggest that concerns regarding kernicterus should not restrict the use of monthly sulfadoxine-pyrimethamine for intermittent preventive therapy during pregnancy. There was no indication that ≥3-dose regimens increased or reduced the risk of stillbirth or neonatal death. The risk of spontaneous miscarriages in G1-G2 women was higher among the 3-dose group (RR = 1.78, P = .046 with fixed-effects models and RR = 1.75, P = .06 with random-effects models). These miscarriages, however, were not associated with the third dose because in 3 of the 4 trials that contributed 80% of the study weight, they occurred before 28 weeks of gestation when the third dose had not yet been provided

(Diakite et al., 2011, Filler et al., 2006, Valea et al., 2010) . In the fourth trial, the risk of miscarriage was 2.0% with a monthly regimen, higher than the 1.1% in the 2-dose group but similar to the 2.3% in a third control group consisting of women randomized to passive case detection only instead of intermittent preventive therapy during pregnancy (Parise et al., 1998).

Since the strategic framework for the control of malaria in pregnancy in sub-Saharan Africa was first developed, at least 3 doses of sulfadoxine-pyrimethamine for intermittent preventive therapy during pregnancy has been recommended by WHO for HIV-infected women or for all women in high-HIV-prevalence areas (>10%) where screening for HIV is not conducted. Some countries, such as Cameroon (Leke and Taylor, 2011), Ghana, Zambia, and Zimbabwe, selected 3 doses of sulfadoxine-pyrimethamine in their policy for all pregnant women, but most other countries, including many high-HIV-prevalence countries, implemented the 2-dose regimen and use cotrimoxazole for HIV-infected women (Van Eijk et al., 2011). However, more recently other countries, including Kenya and Malawi, implemented a monthly regimen among HIV-negative women mainly because of concerns about sulfadoxine-pyrimethamine resistance and for pragmatic reasons to minimize the risk for missed opportunities to deliver a second dose (Gill et al., 2007) and to achieve better alignment with WHO's focused antenatal care schedule (a goal-oriented antenatal care approach consisting of 4 visits providing essential evidence-based interventions). In southern Malawi, this has resulted in a marked increase in the uptake of 2 or more doses of sulfadoxine-pyrimethamine (Kalilani et al., 2011).

Our cumulative meta-analysis showed that, with the accumulation of results from the 4 most recent trials reported since 2010, evidence has emerged that 3-dose or monthly sulfadoxine-pyrimethamine for intermittent preventive therapy during pregnancy was associated with a higher birth weight and lower risk of LBW than the 2-dose regimens among pregnant women in sub-Saharan Africa. These data provide support for the new WHO recommendation that intermittent preventive therapy during pregnancy with sulfadoxine-pyrimethamine be provided at each scheduled focused antenatal-care visit in the second and third trimesters in all settings in which intermittent preventive therapy during pregnancy with sulfadoxine-pyrimethamine is recommended (WHO, 2013). Future research should focus on how best to

implement the updated WHO guidelines for intermittent preventive therapy during pregnancy with sulfadoxine-pyrimethamine (WHO, 2013) and specifically their integration with focused antenatal care. Continued monitoring of the association between population-level sulfadoxine-pyrimethamine resistance and the effectiveness of intermittent preventive therapy during pregnancy is required.

Chapter 5: Parasite clearance following treatment with sulfadoxine-pyrimethamine for intermittent preventive treatment in Burkina-Faso and Mali: 42-day in-vivo follow-up study

5. Chapter 5-Parasite clearance following treatment with sulfadoxine-pyrimethamine for intermittent preventive treatment in Burkina-Faso and Mali: 42-day in-vivo follow-up study

5.1 Introduction

In sub-Saharan Africa, malaria places 32 million pregnancies at risk of maternal anaemia and intrauterine growth retardation resulting in low birth weight (LBW) annually (Dellicour et al., 2010, Desai et al., 2007, Steketee et al., 2001). The World Health Organization (WHO) recommends Intermittent Preventive Treatment in pregnancy (IPTp) with at least two doses of sulfadoxine-pyrimethamine (SP) for the control of malaria in pregnancy (WHO/AFRO, 2004). The 2-dose IPTp-SP regimen has been shown to be very effective and is associated with an average reduction in the risk of LBW of 29% (Ter Kuile et al., 2007). More recent meta-analysis has shown that this can be enhanced further by providing three or more doses of SP during pregnancy (Kayentao et al., 2013).

However, the emergence of SP resistance is potentially reducing the effectiveness of SP. In the early 2000s, SP was abandoned as first line treatment for symptomatic malaria in the general population in sub-Saharan Africa in favour of more effective artemisinin based combination therapy (ACT). Because IPTp with SP continued to provide significant protection in areas with moderate to high parasite resistance (Ter Kuile et al., 2007) SP continues to be recommended by WHO for IPTp, and is currently the only anti-malarial used for this indication (World Health Organization; Global Malaria Program, 2007). The degree of SP resistance correlates with the frequency of single nucleotide polymorphisms (SNPs) that encode amino acid substitutions in the dihydrofolate reductase (*dhfr*) and dihydropteroate synthetase (*dhps*) genes of *Plasmodium falciparum* (Pf) which encode the drugs' target enzymes. High grade resistance is a particular concern in eastern and southern Africa (Naidoo and Roper, 2011), where high frequencies of parasites bearing haplotypes with three mutations in *dhfr* (encoding the N51I, C59R, and S108N) and two in *dhps* (encoding the A437G and K540E substitutions) exist, especially if the additional *dhfr*164L or *dhps*581G mutations occur (Gesase et al., 2009, Harrington et al., 2009). The latter has recently been associated with poor birth outcomes in IPTp-SP recipients (Harrington et al., 2011), although this association has not yet been confirmed in other studies in eastern and southern Africa (Kalilani et al., 2011, Menendez et al., 2011, Taylor et al., 2012).

In contrast, the parasite populations in western Africa seem to be mostly sensitive to SP (Coulibaly et al., 2006, Diakite et al., 2011, Dokomajilar et al., 2006, Naidoo and Roper, 2011), and IPTp-SP has proven to be highly effective and efficacious in clinical trials and observational studies (Diakite et al., 2011, Kayentao et al., 2013, Sirima et al., 2006). However, spread of SP resistance from eastern and southern Africa, or the de-novo development of high-level SP resistance may occur and monitoring of the effectiveness of SP when employed as IPTp is essential.

Despite this need, there are no internationally standardized methods to evaluate the *in vivo* effectiveness of IPTp-SP. Furthermore, the relationship between the level of SP resistance as measured by molecular markers and impact of IPTp-SP on birth parameters, or the treatment response in asymptomatic women receiving SP for IPTp is not known. Hitherto, monitoring SP resistance was predominantly based on *in vivo* treatment responses among symptomatic children with acute malaria. However, extrapolation from children to asymptomatic pregnant women is not appropriate as protective immunity against *P. falciparum* malaria is acquired progressively with cumulative exposure and age. As a result pregnant women in endemic areas remain typically asymptomatic when infected and have lower parasites densities than sick children and as a result have better treatment responses to antimalarials, including SP (Kalanda et al., 2006, Tagbor et al., 2007). We therefore conducted a single arm 42 days *in vivo* efficacy study of SP to determine the parasitological treatment response to SP and the duration of post-treatment prophylaxis among asymptomatic parasitaemic women receiving SP for IPTp in Mali and Burkina Faso. We also assessed the prevalence of molecular markers for SP resistance to correlate with the treatment responses.

5.2 Methods

5.2.1 Study sites and study period

In Mali, the study was conducted from July 2009 to March 2010 in 2 district health centres located in the towns of Kita in the Kayes region in western Mali and in San in the Segou region situated approximately 500 kilometres east of Kita (Figure 5. 1). Malaria transmission in the two sites is typical for most of the Sahel region with highly seasonal transmission restricted to a single period of 3 to 5 months during and shortly after the rainy season, with

peak transmission in October. The degree of SP resistance is low in these areas and the quintuple *dhfr/dhps* haplotype has not been found yet (Diakite et al., 2011), although the *dhps*581G mutation has been described in isolation of other mutations in other settings (Thera et al., 2005, Wang et al., 1997).

In Burkina Faso, the study was conducted from January 2010 to December 2011 in 5 recruitment centres in Ziniaré town, Oubritenga Province, located 400 km South-East of San. Malaria transmission is seasonal peaking in September-October. In 2003, the Polymerase Chain Reaction (PCR) adjusted parasitological failure rate by day-28 was 13% among symptomatic primi-secundigravida with acute *falciparum* malaria in Ouagadougou, 50 kilometers from the study site (Coulibaly et al., 2006).

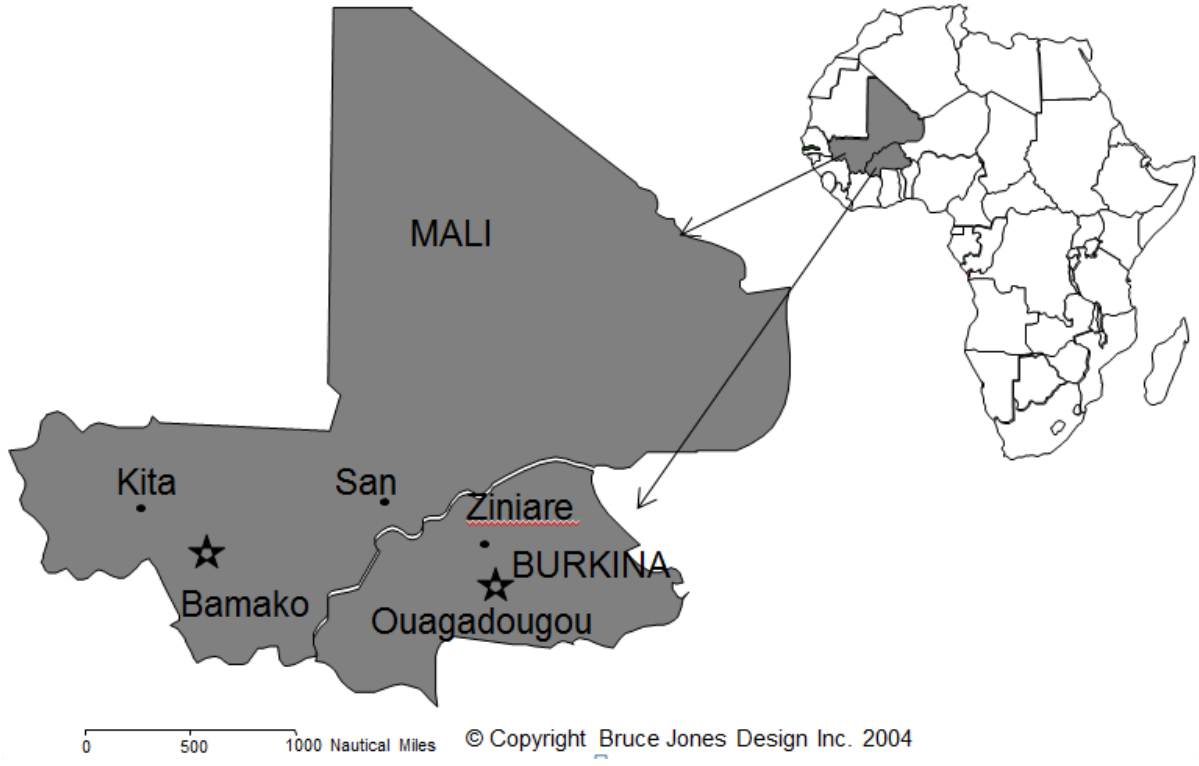


Figure 5. 1 :Study sites: Kita and San for Mali, Ziniare for Burkina-Faso

5.2.2 Participants and procedures

In both countries, pregnant women of all parities with a gestational age between 16-30 weeks attending for antenatal care for their first dose of IPT-SP were included. Women were screened for malaria infection using HRP2 and pLDH-based combo Rapid Diagnostic Tests (RDTs, CareStart™Malaria HRP-2/pLDH[Pf/pan] Combo Test) (Sharew et al., 2009, Maltha et al., 2010). Women with a positive RDT were then screened for malaria parasitaemia by

microscopy and eligible for enrolment if they had a positive blood smear, were asymptomatic, were willing to participate in the 6-week follow-up and provided written informed consent. Women were excluded if they had a history of hypersensitivity to SP or its components, a history of prior use of IPTp-SP during this pregnancy, or a history of receipt of other antimalarials or antibiotics with antimalarial activity in the previous month.

On enrolment, clinical, obstetric and demographic data were obtained and information on bednet type and use recorded. A finger-prick blood sample was taken for malaria smears, haemoglobin assessment, and dried blood spots (DBSs) for parasite DNA.

Three tablets of SP containing a total dose of 1,500 mg sulfadoxine and 75 mg of pyrimethamine were administered as a single dose on day 0 by the study staff. If vomiting occurred within 30 minutes after administration, the full dose was re-administered. Women were scheduled to be seen again weekly from day 7 onwards for 42 days for a brief clinical exam, assessment of the axillary temperature and collection of blood by finger prick for malaria smears, RDT, and DBSs for PCR. Participants were asked to return to the study clinic any time they felt ill in between the scheduled visits. Women with positive smear or severe malaria at any time on or after day 4 were treated according to national guidelines.

In Mali, the study drug used was manufactured by Kinapharma limited Ltd, Ghana and in Burkina Faso this was also from Kinapharma limited Ltd, Ghana and Medreich limited, India. A sample of 50 tablets from each batch was assessed for quality using high-performance liquid chromatography (HPLC) conducted in Atlanta, GA, USA by the US Centers for Disease Control and Prevention (CDC) to determine the amount of the active ingredient and the dissolution profile. Both brands passed the dissolution and content analyses criteria set by the United States Pharmacopeia (USP).

5.2.3 Laboratory methods

Haemoglobin concentrations were measured using HemoCue® (301 System) on days 0, 14, 28 and 42, and on the day of parasite recurrence. Giemsa stained malaria smears were assessed in duplicate and if a discrepancy was found (positive vs negative) the smear was read by a third expert microscopist. Asexual parasites were counted against 300 leukocytes and densities expressed per mm^3 of blood assuming a leucocyte count of 7,500/mm^3. Smears were declared negative if no parasites were detected in 100 high-power fields.

PCR assays were performed in the laboratories of the Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, USA using genomic DNA (gDNA) extracted from dried blood spots (DBSs)stored on Whatman 3MM filter papers. To differentiate between recrudescence and new infection in follow-up specimens with parasite recurrence, a standard method was employed to genotype parasites at the merozoite surface protein-1 (msp-1), merozoite surface protein-2 (msp-2), and glutamate rich protein (GLURP) genes (Taylor, 2013).

To assess the prevalence of genomic markers of parasite SP resistance, genomic DNA from all parasitemic women was pooled by study site (2 in Mali and 1 in Burkina Faso). Fragments of the *dhfr* and *dhps* genes containing the SNPs of interest were PCR-amplified from the pooled gDNA from each site to produce a mixture of gene fragments (Taylor, 2013), and these PCR products were sequenced on a Roche GS Junior next-generation sequencing system.

5.2.4 Study endpoints classification

The primary outcome was the PCR-unadjusted % of patients with parasites recurrence by day 42, defined as a positive diagnostic test for malaria at any visit between days 4 and 42. To define treatment failure, the standard WHO criteria (WHO, 2003) were used.

5.2.5 Statistical analysis

Data were analyzed using STATA v12 and SPSS version 20. The treatment responses are summarized by weeks of follow-up. The therapeutic response was estimated using the Kaplan-Meier product limit formula (WorldWide Antimalarial Resistance Network (WWARN), 2011). In the PCR-unadjusted analysis, recurrences were treated as treatment failures and all other events (e.g. withdrawal or protocol deviations) resulted in censoring at the time of that event, or at the time of their last follow-up visit in case of loss to follow-up. A similar strategy was used for the PCR-adjusted analysis except that patients with new *P. falciparum* infections (reinfections) were censored at the time of parasite reappearance (WorldWide Antimalarial Resistance Network (WWARN), 2011).

5.2.6 Ethical considerations

The protocol was approved by the Faculty of Medicine, Pharmacy and Dentistry, University of Bamako, Mali, the National Ethical Review Committee and Ministry of Health, Burkina-

Faso, the University of North Carolina, United States, and the Liverpool School of Tropical Medicine, United Kingdom.

5.3 Results

5.3.1 Treatment responses

Overall 580 of 584 women who fulfilled all enrollment criteria were enrolled (99.3%, Figure 5.2 & Table 5.1), and 572 of the 580 contributed to the survival analysis. Eight of the 33 women lost to follow-up were not seen after day 0; 3 from Mali and 5 from Burkina-Faso. The remaining 25 were censored on the day they were last seen.

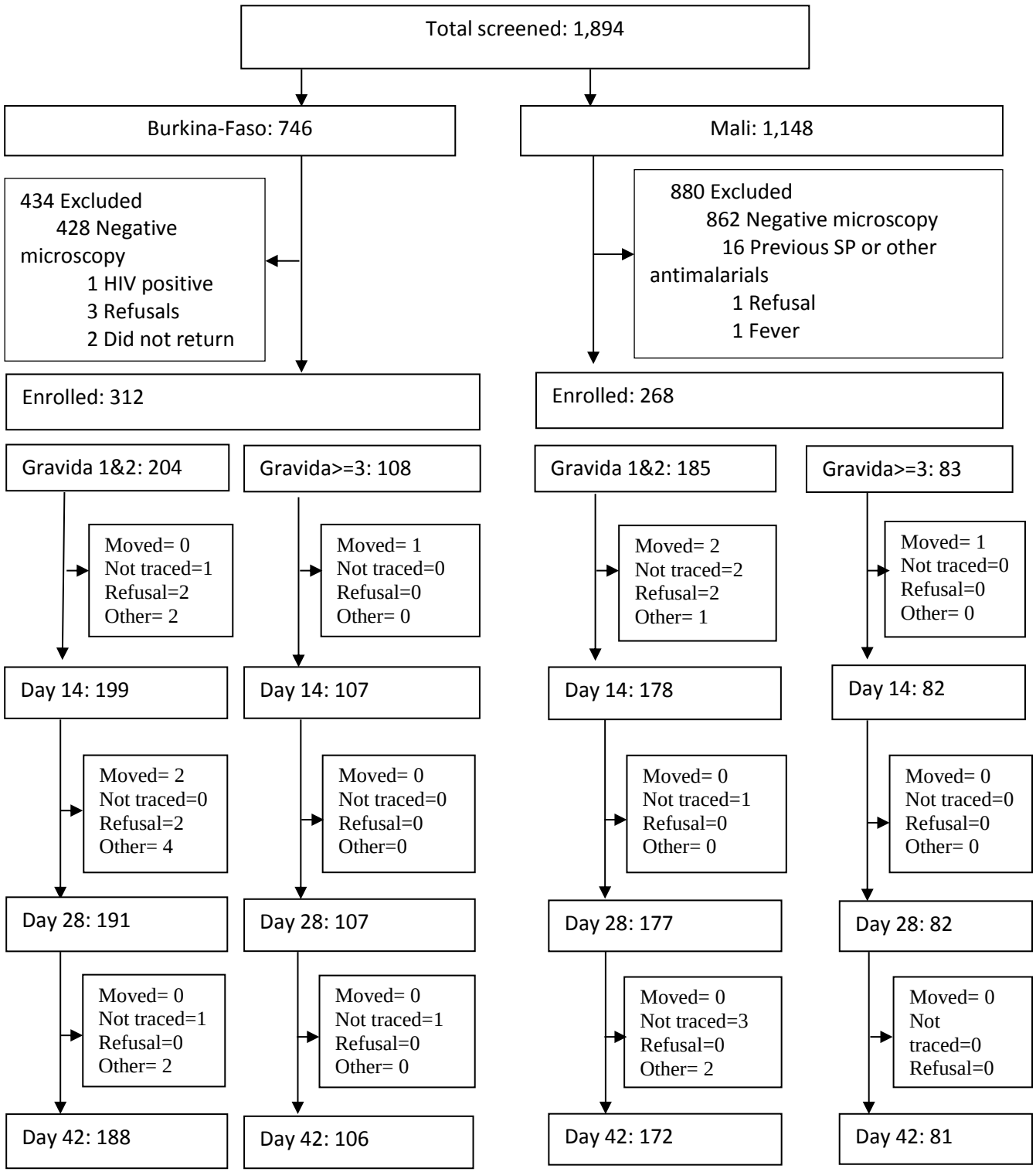


Figure 5.2 : Profile of women screened, enrolled, and completing the study.

Chapter 5

Table 5.1: Baseline characteristics of women enrolled in SP *in vivo* efficacy study, Burkina-Faso and Mali			
	Burkina-Faso N = 312	Mali N =268	All N = 580
Age, years, Mean (SD)	23.6 (5.4)	21.1 (5.1)	22.5 (5.4)
Residing in rural area, n (%)	81 (30.2)	146 (45.2)	222 (38.3)
Knows the date of LMP, n (%)	44 (16.4)	61 (19.6)	105 (18.1)
Pregnancy number Median (range) First or second pregnancy, n (%)	 2 (1-8) 204 (65.4)	 2 (1-9) 185 (69.0)	 2 (1-9) 389 (67.1)
Use of a bed net last night [a] Any net, n (%) ITN, n (%)	 187 (60.1) 171 (55.0)	 207 (77.2) 180 (67.2)	 394 (68.1) 351 (60.6)
Use medicine in first trimester Any medicine, n (%) Antimalarial, n (%)	 6 (1.9) 2 (0.6)	 25 (9.3) 15 (5.6)	 31(5.3) 17 (2.9)
Fundal height, cm Mean (SD)	 21.5 (2.9)	 21.8 (3.2)	 21.7 (3.1)
Gestational age, weeks Mean (SD)	 25.3 (3.1)	 25.4 (3.2)	 25.3 (3.1)
Maternal height, cm Mean (SD)	 162.7 (6.2)	 162.2 (6.3)	 162.4 (6.2)
Maternal weight, kgs Mean (SD)	 57.8 (7.5)	 56.4 (8.5)	 57.2 (8.0)
Haemoglobin, g/dL [b] Mean (SD) Anaemia (Hb<11 /dL), n (%) Moderate-Severe anaemia (Hb<8 g/dL), n (%)	 10.1 (1.4) 225 (72.4) 22 (7.1)	 9.6 (1.6) 198 (80.5) 40 (16.3)	 9.9 (1.5) 423 (75.9) 62 (11.1)
Peripheral parasitaemia GMPD/µl (95% CI)	623 (537-723)	716 (598-859)	664 (592-746)
Notes: Data are numberso (%), unless otherwise indicated. N, sample size; n, number of events; SD, Standard deviation; LMP, Last Menstrual Period; ITN, Insecticide Treated Net; cm, centimeters; kgs, kilograms; g/dL, Gram per deci-Litre; Hb, Haemoglobin; GMPD/µl, Geometric Mean Parasite Density per microliter. [a]Bed net use was not evaluated in 1 subject from Burkina-Faso. [b]Haemoglobin was not measured for 1 subject in Burkina-Faso and 22 in Mali.			

PCR-unadjusted efficacy: Based on microscopy, overall 27 of the 572 women had a recurrence of parasitaemia by the end of follow-up (Mali 8; Burkina Faso 19). The cumulative recurrence risks by day 42 estimated by survival analysis were 4.9% overall, and 3.2% and 6.5% in Mali and Burkina Faso respectively (Hazard Ratio [HR] Burkina vs Mali=2.14, 95% CI 0.93-4.90; P=0.070 (Table 5.2 & Figure 5.3). The recurrence risk was higher among primi - secundigravidae (6.4%) than multi-gravidae (2.2%), HR=3.01 (1.04-8.69; P=0.042) (Figure 5.4).

PCR-adjusted efficacy: From 26 of the 27 recurrences, DNA could be extracted and 24 were genotyped successfully. This suggested that only 6 of the 24 were recrudescences. The PCR-adjusted cumulative failure rate obtained by survival analysis was 1.1% overall, and 0.8% in Mali and 1.4% in Burkina-Faso (Figure 5.3). Overall, median (range) time to PCR-adjusted failure and to reinfection was 21 (7-35) and 35 (7-43) days, respectively.

Haematological response: There was a significant increase in the mean haemoglobin concentrations compared to enrolment at all-time points measured in both countries and both among primi-secundigravida and multigravida (Figure 5. 5 & Table 5.3).

Table 5.2: Parasitological efficacy of SP among women enrolled in Burkina-Faso and Mali						
Characteristics	Burkina-Faso N = 312		Mali N =268		All N =580	
PCR Days	non-adjusted	adjusted	non-adjusted	Adjusted	non-adjusted	Adjusted
Day 7:						
Number at risk	307	307	265	265	572	572
Failures, n (%)						
ETF	0	0	0	0	0	0
LCF	0	0	0	0	0	0
LPF	2 (0.5)	1 (0.3)	1 (0.4)	1 (0.4)	3 (0.5)	2 (0.3)
ACPR n (%)	305 (99.5)	306 (99.7	264 (99.6)	264 (99.6)	569 (99.5)	570 (99.7)
Day 14:						
Number at risk	306	306	260	260	566	566
Failures n (%)						
ETF	0	0	0	0	0	0
LCF	0	0	0	0	0	0
LPF	3 (1.0)	2 (0.7)	1 (0.4)	1 (0.4)	4 (0.7)	3 (0.5)
ACPR n (%)	303 (99.0)	304 (99.3)	259 (99.6)	259 (99.6)	562 (99.3)	563 (99.5
Day 21:						
Number at risk	300	300	259	259	559	559
Failures n (%)						
ETF	0	0	0	0	0	0
LCF	0	0	0	0	0	0
LPF	3 (1.0)	2 (0.7)	1 (0.4)	1 (0.4)	4 (0.7)	3 (0.5)
ACPR n (%)	297 (99.0)	298 (99.3)	258 (99.6)	258 (99.6)	542 (99.5)	556 (99.5)
Day 28:						
Number at risk	297	297	259	259	556	556
Failures n (%)						
ETF	0	0	0	0	0	0
LCF	0	0	0	0	0	0
LPF	7 (2.4)	3 (1.0)	2 (0.8)	2 (0.8)	9 (1.6)	5 (0.9)
ACPR n (%)	290 (97.6)	294 (99.0)	257 (99.2)	257 (99.2)	547 (98.4)	551 (99.1
Day 35:						
Number at risk	295	294[a]	253	254	548	547[a]
Failures n (%)						
ETF	0	0	0	0	0	
LCF	0	0	0	0	0	0
LPF	14 (4.8)	4 (1.4)	2 (0.8)	2 (0.8)	16 (2.9)	6 (1.1)
ACPR n (%)	281 (95.2)	290 (98.6)	251 (99.2)	252 (99.2)	532 (97.1)	541 (98.9)
Day 42:						
Number at risk	293	292 [a]	253	251[a,b]	546	544 [c]
Failures n (%)						
ETF	0	0	0	0	0	0
LCF	0	0	0	0	0	0
LPF	19 (6.5)	4 (1.4)	8 (3.2)	2 (0.8)	27 (4.9)	6 (1.1)
ACPR n (%)	274 (93.5)	288 (98.6)	245 (96.8)	249 (99.2)	519 (95.1)	538 (98.9)
Median (range) time in days	35 (7-43)[d]	21 (7-35)[e]	42 (7-42)[d]	18 (7-29)[e]	35 (7-43)[d]	21 (7-35)[e]
Notes: The table is showing *in vivo* cumulative response to sulfadoxine-pyrimethamine PCR, Polymerase Chain Reaction; ETF, Early Treatment Failure; LCF, Late Treatment Failure; LPF, Late						

parasitological Failure; ACPR, Adequate Clinical and Parasitological Response
[a]One PCR inconclusive
[b]One recurrence of parasites not done for PCR analysis.
[c]PCR inconclusive (1 from Mali, 1 from Burkina) and no PCR analysis (1 from Mali). These three cases were censored in the survival analysis.
[d]Median (range) time to reinfection
[e]Median (range) time to PCR-adjusted failure

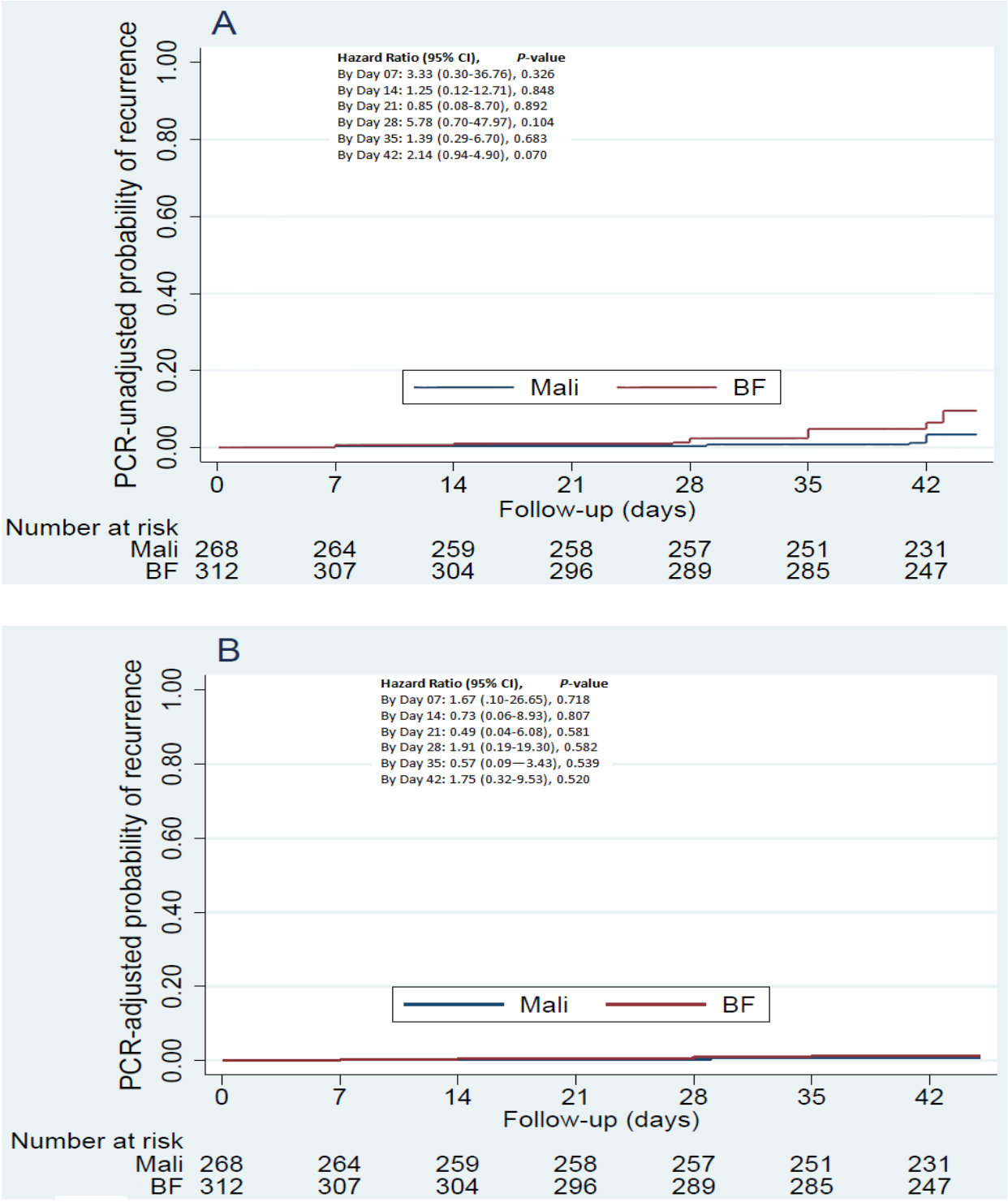


Figure 5.3: Probability of parasitological failure by microscopy in Burkina-Faso and Mali: PCR unadjusted (Panel A) and PCR adjusted (Panel B)

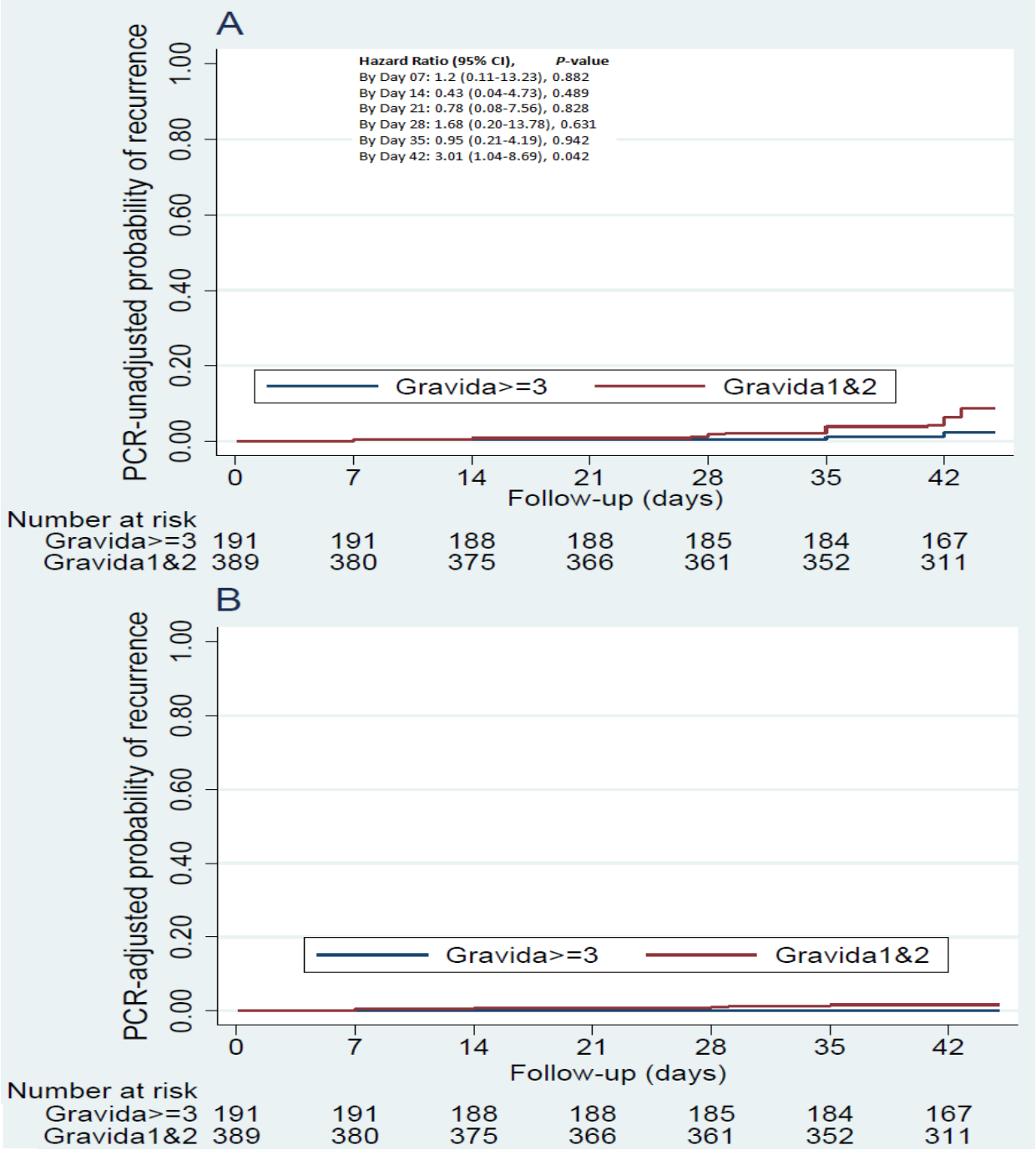


Figure 5.4: Probability of parasitological failure by microscopy by gravida (Gravida1&2, primi-secundigravida; Gravida>=3, multigravida): PCR unadjusted (Panel A) and PCR adjusted (Panel B)

Table 5.3 : Heamoglobin concentration and anaemia among women enrolled in Burkina-Faso and Mali			
Characteristics	Burkina-Faso	Mali	All
Day 0			
N	311	246	557
Mean haemoglobin (SD), g/dl	10.1 (1.4)	9.6 (1.6)	9.9 (1.5)
Anaemia (<11 g/dl), n (%)	225 (72.4)	198 (80.5)	423 (75.9)
Day 14			
N	301	237	538
Mean haemoglobin (SD), g/dl	10.2 (1.3)	10.1 (1.4)	10.2 (1.3)
Anaemia (<11 g/dl), n (%)	208 (69.1)	177 (74.7)	385 (71.6)
Mean difference, 95%CI[a]	0.13 (0.004, 0.26)	0.44 (0.28, 0.59)	0.26 (0.16, 0.36)
Risk Ratio, 95%CI[b]	0.96 (0.86, 1.06)	0.93(0.84, 1.02)	0.94(0.88, 1.01)
Day 28			
N	290	244	534
Mean haemoglobin (SD), g/dl	10.7 (1.2)	10.6 (1.2)	10.6 (1.2)
Anaemia (<11 g/dl), n (%)	171 (59.0)	153 (62.7)	325 (60.7)
Mean difference, 95%CI[a]	0.60 (0.46,0.74)	0.87 (0.69, 1.06)	0.72 (0.61, 0.83)
Risk Ratio, 95%CI[b]	0.82 (0.72, 0.92)	0.78 (0.70, 0.87)	0.80 (0.74, 0.87
Day 42			
N	265	249	514
Mean haemoglobin (SD), g/dl	11.0 (1.2)	10.9 (1.3)	10.9 (1.3)
Anaemia (<11 g/dl), n (%)	127 (47.9)	120 (48.2)	247 (48.1)
Mean difference, 95% CI[a]	0.87 (0.65, 1.09)	1.30 (1.11, 1.49)	1.06 (0.93, 1.18)
Risk Ratio, 95% CI[b]	0.66 (0.57, 0.77)	0.60 (0.52, 0.69)	0.63 (0.57, 0.70)

Notes:
N, sample size; n, number of events; SD, standard deviation; g/dl, gram per decilitre; CI, confidence interval.
[a]Mean difference and 95% confidence interval for each time that haemoglobin was measured using day 0 as reference category.
[b]Risk ratio and 95% confidence interval for each time that haemoglobin was measured using day 0 as reference category, adjusted for gravida and site (all), and for gravida (in each country) .

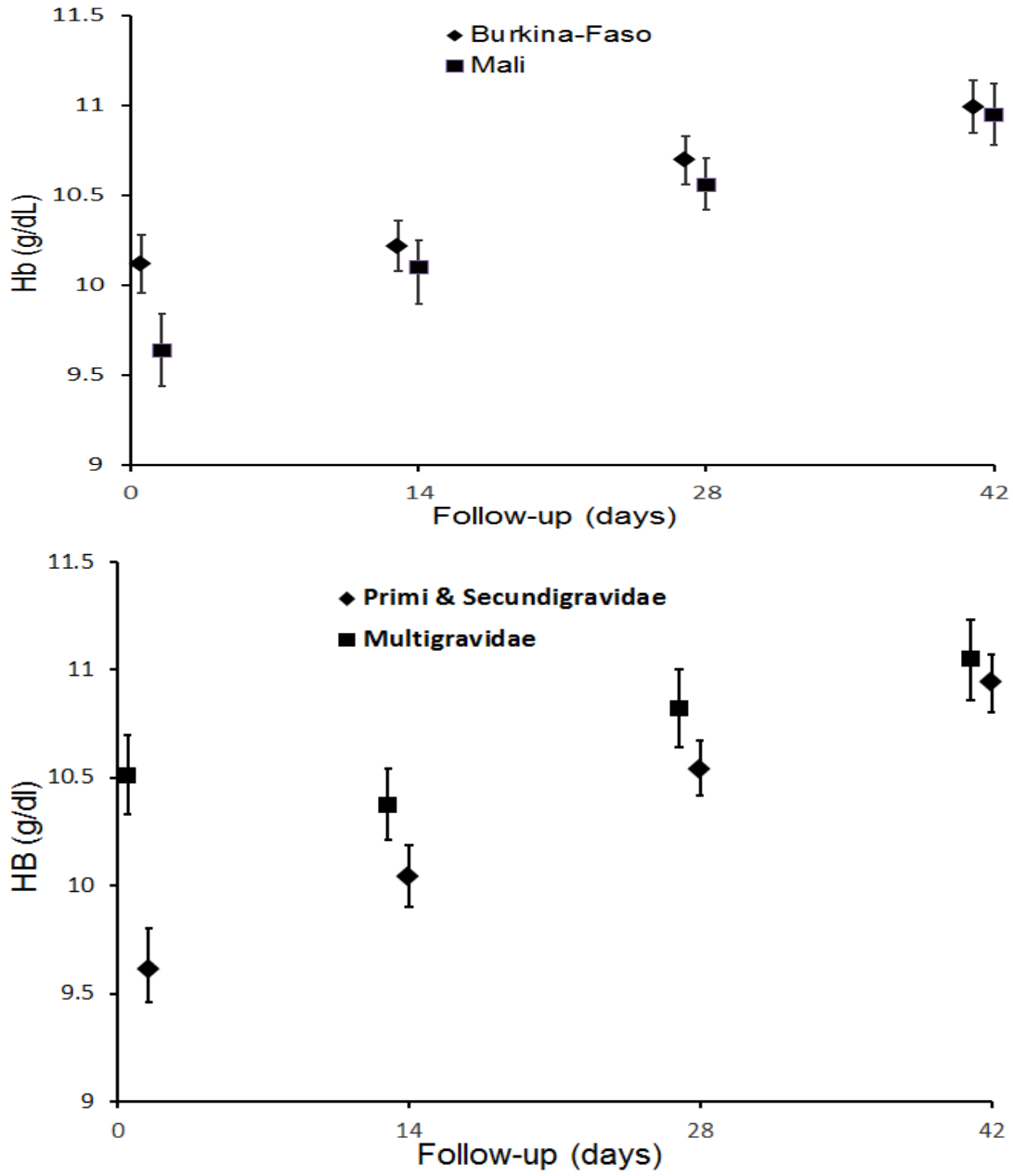


Figure 5. 5: Increase in haemoglobin by country in all gravida (top panel), by gravida (bottom panel).

Analysis was done with repeated measures Generalized Estimating Equation (GEE), adjusted for the baseline haemoglobin levels on Day-0. Black squares or diamonds represent the point estimates and vertical lines the corresponding 95% confidence intervals

5.3.2 Prevalence of molecular markers for SP resistance at booking

No *dhfr* 164L or *dhps*581G mutations were found in any of the three sites; the *dhps* 540E mutation was found in one site of the 2 sites Mali, but at a very low prevalence (Figure 5. 6). Several novel non-synonymous mutations in *dhps* were detected; in Kita, a V452I substitution was present in 1.43 % (1.28 – 1.59) of reads and a L590H substitution in 1.05% (0.78 – 1.32). In Ziniare, an R532S substitution was present in 1.02% (0.78 – 1.26) of reads, while a G533R substitution was present in 1.94% (1.61 – 2.27) (data not shown).

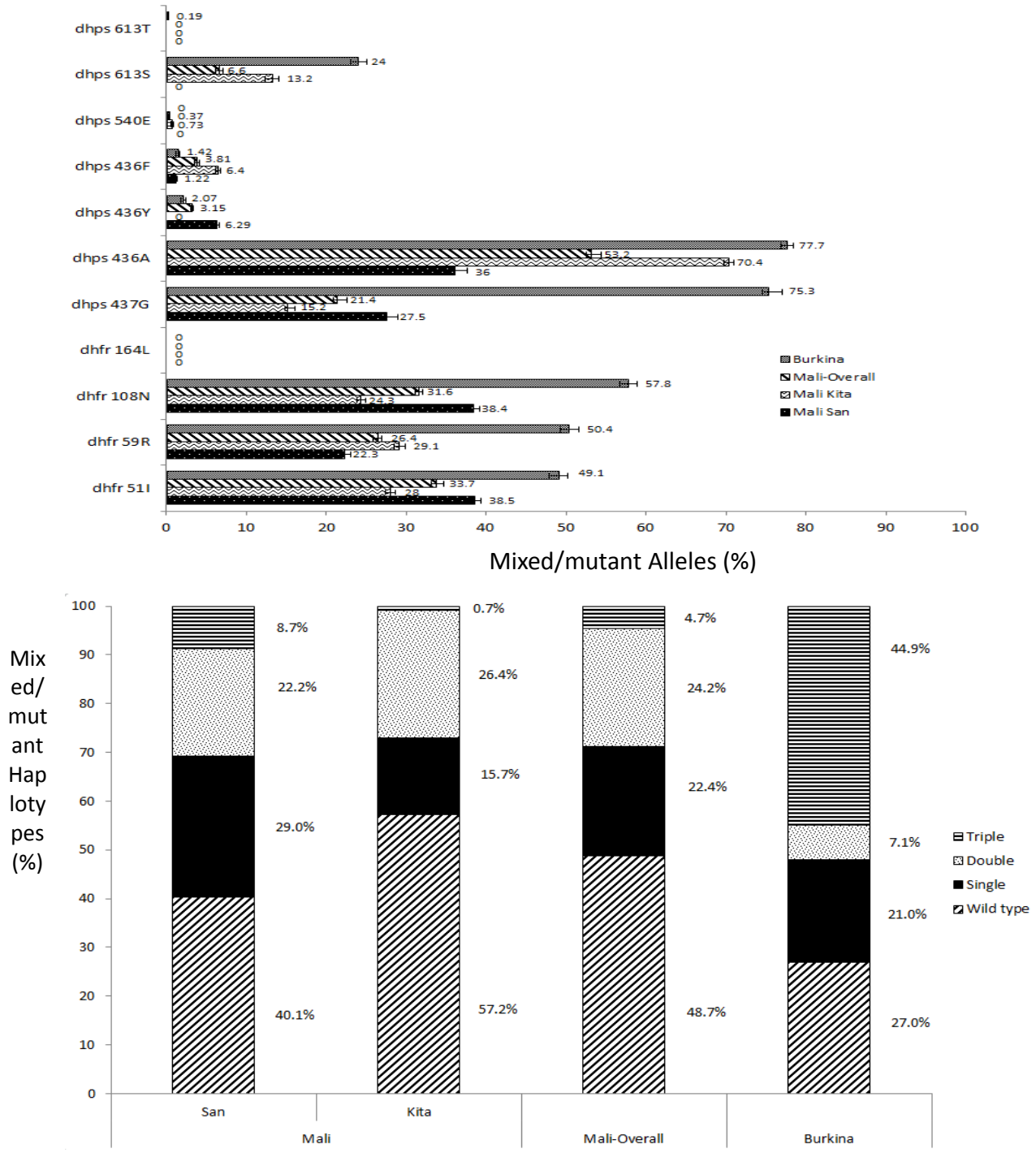


Figure 5. 6: Prevalence of SP resistance molecular makers in Burkina-Faso and Mali among women during antenatal booking; *dhfr* /*dhps* alleles (Top panel) and *dhfr* haplotypes (Bottom panel)

5.4 Discussion

SP when given as IPTp to asymptomatic parasitaemic pregnant women was associated with a high cure rate and marked increases in haemoglobin concentrations by day 42 in the 3 study sites in Mali and Burkina-Faso. Overall, only 4.9% of women had a recurrence of parasites by day 42, and genotyping suggested that the vast majority of these were reinfections. Overall only 1.1% of treatments resulted in true treatment failures (recrudescence) and all of these were asymptomatic.

The pooled molecular assays for the surveillance of SP resistance showed that almost 50% of the parasite population in Burkina Faso, but only 9% in San and <1% in Kita, carried the *dhfr* triple mutations. The pooled deep sequencing of *Plasmodium falciparum* parasitemias can provide estimates of the mutant allele frequencies, but does not provide estimates of the quadruple and quintuple *dhfr/dhps* haplotypes. Nevertheless, the *dhps* 540E mutation, which is a proxy for the quintuple haplotype conferring mid-level resistance to SP, was present in only one of the two sites of Mali and at very low frequency (0.73%, 95% CI 0.58-0.87). The mutation at *dhfr* codon 164L and *dhps* codon 581G conferring very high-level resistance to SP were absent. In addition, there were several novel mutations in *dhps* which were limited to a very low frequency. Their clinical and biological significance is unknown, but their quantification underscores the ability of the pooled genotyping approach to uncover low-level subpopulations of parasites.

The 1.4% failure rate in Burkina Faso among asymptomatic women in this study is in contrast to the 13% PCR-adjusted failure rate by day-28 observed in the previous in-vivo study among symptomatic pregnant women conducted in 2003 in an area located ≈32 miles south from the current site (Coulibaly et al., 2006). The average parasite densities in the previous study were 10 fold higher than in the current study, illustrating the differences in treatment responses when SP is used as IPTp in asymptomatic women with predominantly low-grade parasitaemia vs. acutely ill women requiring case-management drugs. This may in part explain the earlier findings from randomized controlled trials that IPTp-SP remained surprisingly effective in areas with moderate to high levels of SP resistance (Kayentao et al., 2013, Ter Kuile et al., 2007).

Our observations provide an important contribution to the understanding of the predictive value of the frequency of population estimates of the different *dhfr* and *dhps* mutations on

the efficacy of SP in clearing malaria infection among asymptomatic pregnant women, especially when our results are compared against day 42 failure rates in areas with higher resistance. For example, the *dhfr* triple mutation (Ile51+Arg59+Asn108) was present in almost 50% of the parasite population in Burkina Faso yet only 1.4% of the treatments recrudesced by day 42. The *dhfr* triple mutation is known to confer intense pyrimethamine resistance in vitro (Gregson and Plowe, 2005) and is associated with an approximate 1,000-fold reduction in pyrimethamine susceptibility (White, 2005) and with an increased risk of SP treatment failure in children with acute malaria (Kublin et al., 2002, Mockenhaupt et al., 2005, Picot et al., 2009). These combined data suggests that parasite densities and immunity contribute importantly to parasite clearance, which in turn influences the association of treatment outcome with *dhfr and dhps* alleles.

It is likely that the results of this study are representative for large parts of West and Central Africa that have a similar low geographic prevalence of the *dhfr/dhps* quadruple or quintuple mutations reflecting low and mid-level resistance to SP (Naidoo and Roper, 2011). A key question is whether this situation can be sustained or whether further development of SP drug resistance is inevitable in this region. Mutations arise under antifolate pressure in a stepwise fashion, with successive mutations conferring higher levels of resistance (Cortese et al., 2002). Previous studies from Ghana showed a rapid increase in the prevalence of the triple-mutant *dhfr* alleles among *falciparum* isolated from pregnant women in an area where pyrimethamine prophylaxis (as mono-therapy) was used 6 to 8 years previously for the prevention of malaria (Mockenhaupt et al., 2008). Some fitness-reducing mutations, such as the *dhfr* I164L can only be sustained under conditions of sustained drug pressure. The switch from SP as first line treatment for symptomatic malaria in the general population to ACTs will have a marked impact on reducing SP drug pressure in the population (Malisa et al., 2010). Modelling of the impact of the introduction of IPTi in infants suggest that use of SP in small target populations such as infants or pregnant women may not sustain sufficient drug pressure to impact on the spread of drug resistance. This was also suggested in field studies in Mali (Dicko et al., 2010). However, many West African countries including Mali and Burkina are seeking to implement Seasonal Malaria Chemoprevention (SMC) (WHO, 2012) in children which would provide presumptive treatment over the course of the transmission season to a much larger fraction of the population. Although, the combination of

amodiaquine and SP is one of the main candidate antimalarials for SMC, it is unclear if the introduction of this strategy will indeed increase SP drug pressure. The effect of SMC on drug pressure may be minimal if implemented on a large enough scale to impact on malaria transmission and the total parasite biomass in the SMC population, especially if ACTs are used as case-management for clinical episodes caused by any SP resistant parasites that may have escaped the drug action of SMC. It will be clearly important to monitor the prevalence of molecular markers of parasite resistance to SP as part of any SMC policy that uses combination therapy containing SP.

Our investigation found a high prevalence of anaemia and showed that SP treatment was associated with a marked increase in mean haemoglobin levels by day 42. The fact that the impact was most pronounced in the primi- and secundigravidae, the group most susceptible to adverse effect of malaria, may indicate that even these asymptomatic infections are an important cause of maternal anaemia in this subgroup. These findings are consistent with previous findings that showed IPTp has a marked beneficial impact on moderate-to-severe anaemia in Mali (Kayentao et al., 2005).

Our study was limited by the lack of genotyping of parasites from individual women for molecular markers of SP resistance, and the genomic DNA from pooled sequencing by study site was not able to explore the correlation between treatment efficacy or the haematological response in individual women and SP resistance molecular markers.

5.5 Conclusion

This is among the first studies to examine the 42-day *in vivo* response of IPTp-SP in asymptomatic women in areas with low level of SP resistance in West Africa. Despite growing concerns about the impact of SP resistance in eastern and southern Africa, this study shows that SP remains effective at clearing existing infections and improving haemoglobin concentration when provided as IPTp to asymptomatic pregnant women in Mali and Burkina-Faso. SP has many attributes that makes it an excellent candidate for IPTp, and it is thus likely that it could remain the drug of choice for IPTp in this region for the foreseeable future. However continued monitoring of SP resistance over the next years in this region coupled with monitoring of IPTp-SP effectiveness on birth parameters is essential.

Chapter 6: Effectiveness of intermittent Preventive Therapy for the Control of Malaria in Pregnancy in five health districts of Mali: an observational study

6. Chapter 6-Effectiveness of intermittent Preventive Therapy for the Control of Malaria in Pregnancy in five health districts of Mali: an observational study

6.1 Introduction

In sub-Saharan Africa, approximately 32 million pregnancies are at risk of acquiring *P. falciparum* infections each year (Dellicour et al., 2010) causing maternal anaemia and low birth weight (LBW) (Desai et al., 2007, Steketee et al., 2001).

Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) is recommended by the World Health Organization (WHO) for reducing the risks of adverse birth outcomes associated with malaria in pregnancy and this strategy has been shown to be very effective in controlled trials across different malaria endemic regions in sub-Saharan Africa (Kayentao et al., 2005, Njagi et al., 2003, Parise et al., 1998, Shulman, 1999, Ter Kuile and Steketee, 2007). Implementation of this strategy began in East-Africa in the 1990s followed by West-African countries in the 2000s. To date, IPTp in combination with insecticide treated nets (ITN) is policy in 37 countries of sub-Saharan Africa (Van Eijk et al., 2011).

In Mali, IPTp-SP was first introduced for pilot implementation in few districts in 2003 by the Ministry of Health with support of UNICEF. In 2006, a demographic health survey (DHS) suggested the coverage of 2 doses SP to be only 4% despite the efforts deployed by UNICEF and the international children's charity “Save the Children” (DHS, 2006), but this had increased to 19.9% in 2012 following further support from the Global Fund and other donors to the National Malaria Control Program (DHS, 2012-2013) . Similar efforts to increase the uptake of IPTp-SP are under way across Africa (van Eijk et al., 2013).

However, increasing resistance to SP is threatening the effectiveness of this important strategy, especially in eastern and southern Africa (Naidoo and Roper, 2011, Harrington et al., 2011). In western Africa, *P. falciparum* has remained largely sensitive to SP (Flegg et al., 2013) and IPTp-SP appears to maintain good effectiveness in improving birth outcomes in a series of studies in this region (Diakite et al., 2011, Valea et al., 2010, Naidoo and Roper, 2013, Sirima et al., 2006, Vanga-Bosson et al., 2011). However, SP resistance has continued to increase and there are no recent reports assessing its effectiveness under programmatic conditions.

Chapter 6

Here we report the results of 6 observational cross-sectional studies conducted between 2006 and 2010 among women attending for delivery in 5 districts with different levels of malaria transmission in Mali. The surveys were conducted to assess the uptake and effectiveness of IPTp-SP in a context of high ITN use and low population levels of SP resistance as determined by molecular markers (Dicko et al., 2010). The results also contribute to a larger pool of similar studies designed to support the development by WHO of a practical standardized protocol for monitoring the impact of SP resistance on IPTp-SP effectiveness in pregnant women in sub-Saharan Africa.

6.2 Methods

6.2.1 Ethics statement

Before enrollment, women were asked to provide written informed consent after the study procedures were explained in their local language (Bambara). The study protocol was approved by the research ethics committees or institutional review boards of the Faculty of Medicine, Pharmacy, and Dentistry of Mali (FMPOS) (all surveys), the Centers of Diseases Control and Prevention (CDC) (for the first 4 surveys), and the Liverpool School or Tropical Medicine (LSTM) (last 2 surveys).

6.2.2 Study sites

The study consisted of 6 surveys conducted between September 2006 and January 2010 in 5 sites in 5 different health districts across Mali, namely Koro, Djenne, Bougouni, Kita (1 survey each), and San (2 surveys). These 5 districts were the initial pilot implementation districts for IPTp in 2003-2004 and received specific support from UNICEF and Save the Children. The uptake of IPTp and ITNs is therefore probably higher in these pilot districts than in other parts of Mali. In all 5 sites malaria transmission is highly seasonal with single transmission season peaking between September-October (Figure 6.1). The length of the transmission season is longest in the South where Bougouni is located, lasting 4 to 6 months and starting around June. Further north, the transmission season gets progressively shorter and starts later following the northward migration of the Sahel rainfall pattern typical for this region.

Chapter 6

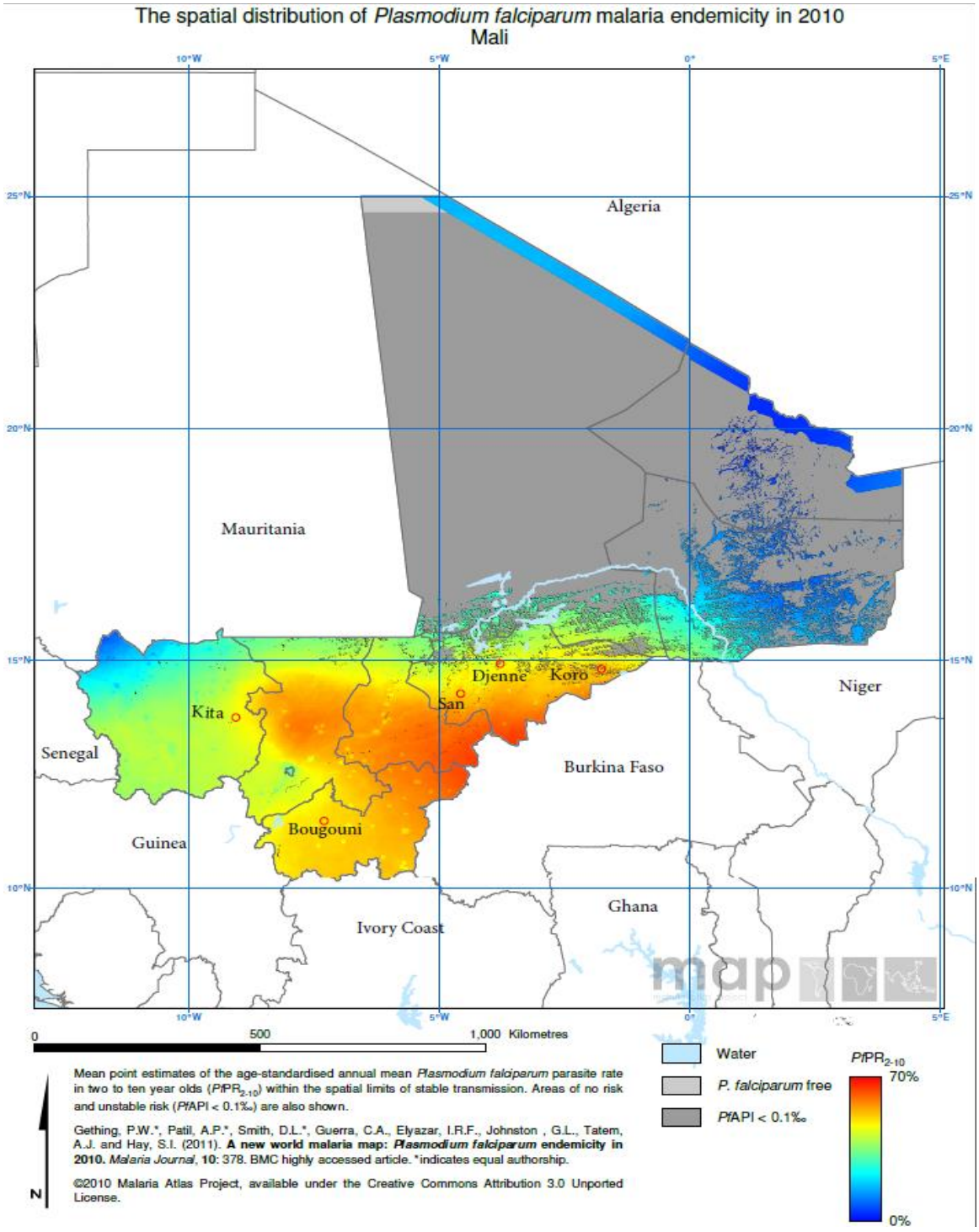


Figure 6.1: Map of different study location in Mali

Source: This map is a product of the MAP project(http://www.map.ox.ac.uk/client_media/pdf/Pf_mean_2010/Pf_mean_2010_MLI.pdf)

Notes: Open circles depict the location of the 5 survey sites

6.2.3 Clinical procedures

All 6 cross-sectional surveys were designed to evaluate the effectiveness of IPTp on birth parameters among all gravida aged 15 years and older. A woman was screened in the delivery unit and if informed consent was obtained an enrolment questionnaire was filled to obtain socio-demographic characteristics, information on the history of fever, and use of ITNs and IPTp. The number of reported SP doses used during pregnancy was verified using antenatal clinic cards or any other ANC records available. The axillary temperature was measured and a maternal capillary blood sample was taken for haemoglobin and malaria smears (thin and thick) and dried blood spots for polymerase chain reaction (PCR) based analyses. Placental blood was collected from the maternal side of the placenta for thick smears and dried blood spots for PCR analysis. Umbilical cord blood was also taken for thick blood smears. Within 24 hours of delivery, singleton neonates were weighed using the same brand of digital scale at each site. Gestational age was assessed using a standardized Ballard examination (Ballard et al., 1979).

6.2.4 Laboratory procedures

Thick blood smears were stained with 4% Giemsa for 20 minutes and examined for malaria parasites. Parasite densities were counted against 300 leucocytes and expressed using an assumed leukocyte count of 7,500 leukocytes /mm^3 of blood. Smears were considered negative if no parasites were detected after counting 100 high power fields. For quality control, 10% of positive and 10% of negative slides were randomly selected and read by an expert microscopist from the Malaria Research and Training Centre (MRTC) in Bamako. Thin smears were used for parasite species diagnosis. A HemoCue machine® (Hemoglobin AB, Ångelholm, Sweden) was used to assess maternal haemoglobin concentrations.

6.2.5 PCR

DNA extraction: A simple DNA extraction method for filter paper strips was used (Djimde et al., 2001). Briefly, approximately 1 x 2 mm piece of blood-soaked filter paper was placed in 50 µl of methanol for 15 min. The methanol was poured off and the paper heated at 95-100^{o} C in 50 µl of water for 10 min. The resulting solution was used as a PCR template. Samples that failed to yield PCR amplification with the methanol method were re-extracted using an

alternative Chelex-based method (Plowe et al., 1995). For the last two surveys the Chelex-based method was used for all samples.

Real time PCR for parasite detection: For the last two surveys (two sites in 2009, (Table 6.1)), parasites were detected in the maternal (antenatal booking and delivery), placental and cord blood using real-time PCR cascade testing approach as previously described (Taylor et al., 2011a). Briefly, parasite DNA was pooled in groups of 4 and tested in a real-time PCR assay targeting all species of Plasmodia. Those demonstrating amplification were re-tested individually in the same assay, and those with positive amplification were then tested in a second real-time PCR assay that differentiates between Plasmodia sp. Those demonstrating amplification in this *P. falciparum* assay were considered positive. All assays were performed in duplicate with appropriate negative and positive controls.

Chapter 6

Table 6.1: Site and socio demographic characteristics and malaria prevention measures in women who delivered in the five study sites							
Characteristics	Bougouni (N=424)	Djenne (N=424)	Koro (N=424)	San (1) (N=424)	Kita (N=615)	San (2) (N=471)	Total (N=2782)
Year of study conduct	Sep06-Mar07	Sep06-Mar07	Sep06-Mar07	Sep06-Mar07	Jul09-Jan10	Jul09-Jan10	
P.f. prevalence in children 2-9 yrs	52.6 [a]	50.5 [a]	48.2 [a]	64.7 [a]	39.5 [a]	66.6 [a]	
SP resistance							
By modelling or pool deep sequencing in unknown population (first four surveys), and in pregnant women before first SP dose (last two surveys)							
DHPS A437G	33.4[a]	32.7 [a]	44.8 [a]	32.6 [a]	15.2 [b d]	27.5 [b d]	
DHPS K540E	0.20 [a]	0.00 [a]	0.11 [a]	0.10 [a]	0.73 [b d]	0 [b d]	
*By nested PCR or pool deep sequencing, women at delivery**							
DHPS A437G	29/45 (64.4) [c]	18/27 (66.7) [c]	24/28 (85.7) [c]	36/55 (67.3) [c]	219/1854 (15.7) [b]	124/428(29.0) [b]	
DHPS K540E	0 [c]	0 [c]	0 [c]	0 [c]	0 [b]	0 [b]	
DHFR triple**	25/38 (65. 8)[c]	5/18 (27.8)[c]	12/24 (50.0)[c]	21/44 (47.7) [c]	0.66 [b]	8.7 [b]	
DHPS/DHFR quadruple **	16/38 (42.1)[c]	4/18 (22.2)[c]	7/18 (38.9)[c]	15/44 (34.1) [c]			
Age years							
Mean (SD)	24.24 (6.79)	26.04 (6.55)	25.34 (6.97)	25.13 (6.13)	24.47 (6.97)	26.45 (6.95)	25.24 (6.80)
Age <20, n/N (%)	67/424 (15.8)	26/424 (6.1)	56/424 (13.2)	38/424 (9.0)	102/615 (16.6)	19/471(4.0)	308/2782 (11.1)
Living in rural area	71/424 (16.8)	116/424 (27.4)	131/424 (30.9	19/424 (4.5)	173/609 (28.4)	84/469 (17.9)	594/2774 (21.4)
Season delivery Rainy, n/N (%)	410/424 (96.7)	233/424 (55.0)	248/424 (58.5)	424/424 (100)	601/615 (97.7)	455/471 (96.6)	2,371/2,782 (85.2)
Median number of pregnancies (range)	3(1-12)	4(1-15)	3(1-15)	3(1-10)	4(1-12)	4(1-13)	3(1-15)
G1-G2, n/N (%)	187/424 (44.1)	149/424 (35.1)	176/424 (41.5)	185/424 (43.6)	165/615 (43.1)	181/471 (38.4)	1143/2782 (41.10)
Bed net during pregnancy, n/N (%)***	239/424 (56.4)	416/424 (98.1)	338/424 (79.7)	371/424 (87.5)	558/613 (91.0)	455/470 (96.8)	2377/2779 (85.53)
ITN last night	152/424 (35.9)	288/424(67.9)	249/424(58.7)	260/424 (61.3)	542/613 (88.4)	444/470 (94.5)	1935/2779 (69.63)

SP, n/N (%)						**Table 6.1 continued**	
0	111/424 (26.2)	154/424 (36.3)	180/424 (42.5)	147/424 (34.7)	155/611 (25.4)	128/470 (27.2)	875 /2777 (31.51)
1	187/424 (44.1)	119/424 (28.1)	147/424 (34.7)	186/424 (43.9)	132/611 (21.6)	175/470 (37.2)	946/2776 (34.1)
2	126/424 (29.7)	151/424 (35.6)	97/424 (22.9)	91/424 (21.5)	322/611 (53.0)	157/470 (33.4)	946/2777 (34.1)
3	0	0	0	0	0	9/470 (1.9)	9 /2777 (0.3)
4	0	0	0	0	0	1/470 (0.2)	1/2777 (0.04)
median time between 1st and 2nd dose-IPTp (range), N, days	58 (18-134), 125	61 (8-166), 151	61 (17-148), 97	59 (8-154), 90	25 (6-141), 311	35 (4-154), 167	42 (4-166), 941
median time between last dose and delivery (range), N, days	74 (8-193), 306	65 (2-172), 270	60 (1-172), 240	80 (1-213), 275	72 (6-141), 440	80 (6-193), 342	72 (3-190), 1873

Notes: N, sample size; n, number of events; SD, standard deviation; G1-G2, first and second pregnancy; ITN, insecticide treated net; SP, sulfadoxine-pyrimethamine; IPT, intermittent preventive treatment in pregnancy; g/dl, gram per decilitre

[a]Prevalences obtained by modelling : Malaria transmission intensity from the published malaria project estimates for 2007 (first 4 surveys) and 2010 (last 2 surveys) of the *P. falciparum* parasite prevalence in children aged 2-10 years (Gething et al., 2011, Hay et al., 2009) and *Pf*dhps (unknown source of sample) assessed by high throughput sequencing-specific oligonucleotide probe-based method (Alifrangis et al., 2005, Lynch et al., 2008, Pearce et al., 2003)

[b]Obtained by pool deep sequencing (Taylor et al., 2013)

[c]Assessed by nested mutation-specific PCR (Diourte et al., 1999)

[d]Sample from IPTp recipients at antenatal clinic visit booking (pre-SP dosing)

*Maternal peripheral blood sample at delivery

**DHFR triple include mutant allele at DHFR codons N51I, C59R, and S108N; DHFR/DHPS quadruple include mutants for DHFR triple + mutant allele at DHPS codon A437G.

***Use of all types of bednets including treated and non-treated

Determination of dhfr and dhps genotypes

To determine the degree of resistance to SP in the population, mutations in *Plasmodium falciparum* dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps) were assessed by PCR amplification and sequencing using maternal blood samples collected at delivery. During the first 4 surveys, nested mutation-specific PCR and/or PCR-RFLP were performed (Diourte et al., 1999). DHFR mutations at codons 108, 51 and 59 and the DHPS mutations at codons 437 and 540 were analysed. During the last two surveys, pooled deep sequencing of *Plasmodium falciparum* isolates was used to quantify the mutant allele frequencies in the *Pfdhfr* and *dhps* in mixed isolates using parasite DNA from 130 samples in San and 117 in Kita that had previously tested positive for *P. falciparum* using rtPCR. The pooled DNA was PCR amplified at the gene, and PCR products were sequenced using a second-generation sequencing platform, which provided the frequency of the drug-resistance mutation in the parasite population (Taylor et al., 2013) (Table 6.1) .

Because use of SP during pregnancy may select for resistant mutations, we also determined the degree of resistance in samples collected from pregnant women at antenatal booking; i.e. before the first dose of SP. These samples were collected from pregnant women in the same study site and period. This information was only available for the last two surveys (San and Kita). For the first 4 surveys, data on the location and time specific population prevalence of mutations in the *pfdhps* /*pfdhfr* genes was obtained from spatiotemporal mathematical modelling courtesy of the World Wide Antimalarial Resistance Network (Flegg et al., 2013).

6.2.6 Definition

LBW was defined as a live singleton weight of <2500 grams, and prematurity as gestational age of <37 weeks by Ballard examination. Anaemia was defined as an Hb level of <11g/dL, and severe anaemia as Hb level <8 g/dL. Small for gestational age (SGA) was determined as <10th percentile in birth weight for attained gestational age (Landis et al., 2009).

Malaria transmission intensity data was obtained using the published Malaria Atlas Project (MAP) estimates for 2007 and 2010 of the *P. falciparum* parasite prevalence in children aged 2-10 years (Gething et al., 2011, Hay et al., 2009).

6.2.7 Sample size calculation and statistical approach

For the first four surveys, the sample size had been calculated based on a study conducted by Kayentao in Mali (Kayentao et al., 2005) which found a placental malaria prevalence of 25% among women who had received 2 doses of IPT with SP. We estimate that the prevalence of placental malaria in the subsequent studies would be slightly lower at 20% because of the anticipated high ITN coverage in these populations. It was calculated that a sample of 385 women was needed per study site to detect a prevalence of 20% with a precision of 4%, and 424 women to allow for 10% of missing data. Because smaller sample sizes were required to detect the proportion of anaemia and low birth weight with similar precision, 424 women was considered to be sufficient for multiple endpoints in each study site.

For the last two surveys, placental malaria, was also used as primary outcome because it is the most malaria-specific end-point and more likely to reflect changes of SP-resistance. The sample size at delivery was based on detecting a 2-fold difference in placental malaria in primi-and secundi gravida who have received the full 2 course of SP versus women who received <2 courses of IPTp (e.g. 10% vs 20%, with 80% power and 95% confidence). If the coverage of 2 course IPTp-SP was very high (>67%) or very low (<33%), enrolment was to be stratified by the history of the number of doses of IPTp received based on their history and antenatal clinic records, to obtain at least a 2:1 or 1:2 ratio of women who received at least 2 courses versus less courses. Sample sizes ranging from 948 to 1103 were needed in settings where placental malaria is less common, e.g. 5% in women receiving 2 dose IPTp and 10% in women receiving less than 2 doses (see table below). Thus based on the local circumstances at least 438 and at most 1103 deliveries would need to be included per site.

Sample size calculations to detect 2-fold difference in placental malaria (5% in women receiving 2+ course and 10% in the <2 courses of IPTp-SP)					
coverage of IPTp			Sample size		
Coverage Ratio	2+ courses	<2 courses	2+ courses (n)	<2 courses (n)	Total N
1:1	50.0%	50.0%	474	474	948
4:1	80.0%	20.0%	1092	273	1365
3:1	75.0%	25.0%	888	296	1184
2:1	66.7%	33.3%	682	341	1023
1:2	33.3%	66.7%	368	735	1103
1:3	25.0%	75.0%	332	995	1327
1:4	20.0%	80.0%	313	1253	1566

Data entry was done using Access 2000 (Microsoft office) and data analysis using Stata 12.0 (StataCorp LP, Texas, USA). Site specific estimates of the effect of IPTp doses on a range of dichotomous outcomes (0, 1, 2) were obtained using log binomial regression in Stata's generalized linear models (GLM) command and expressed as the adjusted prevalence ratio (aPR) and 95% CI. If models did not converge the "difficult" or "binreg" options were used (Cummings, 2009). In all the models, the variable for IPTp dose (0, 1, 2 doses) was forced into the model as the primary exposure variable of interest. Other variables considered for inclusion as possible confounders included location (rural versus urban), age (<18 versus >=18 years), season of delivery (dry versus rainy), gravida group (G1-G2 versus G3+) and ITNs (use versus none use). The delivery endpoints were compared between women who received 2 or more doses of IPTp versus those who received only one course of IPT, or no IPT (e.g. because they did not attend ANC).

6.3 Results

6.3.1 Characteristics

A total of 2,782 women were recruited at delivery during the 6 surveys in the 5 districts. Their mean (SD) age was 25.2 (6.8) years and 11.1% were younger than 18 years old. The median (range) gravida number was 3 (1-15) and 41.1% were primi or secundigravidae. Overall, 2,377 (85.5%) used a bednet and for 1,935 (69.6%) of them this was a treated net used the night before delivery. Overall, 10 women (0.3%) received 3 or 4 doses (1 site), 946 (34.1%) received two, a further 946 (34.1%) one dose, and 875 (31.5%) received no SP (Table 6.1). Among the 1,902 women who received IPTp-SP, the median time between first and second dose was 42 days and that between last dose and delivery was 72 days. The prevalence of anaemia and severe anaemia was 56.5% and 10.8%, respectively with similar risks observed in all the sites. The mean birth weight (SD) among singleton live births was 3,094 (512) grams and 8.2%, 1.9% and 30.1% were classified as LBW, preterm and SGA, respectively, with similar risk across the surveys (Table 6.2).

The prevalence of maternal and placental parasitemia was 18.6% and 16.2%, respectively, and these differed significantly between sites ($p<0.001$ for both) (Table 6.2). The median time between the last dose of SP and delivery was just over 10 weeks (72 days), and this was approximately 11 weeks (78 days) in women with placental malaria vs 71 days in women without evidence of infection at delivery (P=0.067)

Parasite detection PCR data was available for the last two surveys only, both conducted in 2009. The prevalence of *P. falciparum* infection detected by PCR was 3 times (maternal), ≥ 5 times (placental), and 50 times (cord blood) higher than the estimates obtained by microscopy (Table 6.2).

Table 6.2: Clinical and biological characteristics of women who delivered in the five study sites #

Characteristics	Bougouni (N=424)	Djenne (N=424)	Koro (N=424)	San (1) (N=424)	Kita (N=615)	San (2) (N=471)	Total (N=2782)
Axill temp >=37.5°C, n/N (%)	35/424 (8.3)	32/424 (7.6)	45/424 (10.6)	136/424 (32.1)*	43/611 (7.0)	16 /469 (3.4)	307 / 2776 (11.1)
Haemoglobin in g/dl							
Mean (SD)	10.69 (2.03)	10.36 (2.13)	10.55 (2.16)	10.16 (2.00)	10.61 (2.00)	10.53 (1.75)	10.48 (2.01)
Hb<11 g/dL, n/N (%)	210/424 (49.5)	239/424 (56.4)	239/424 (56.4)	286/424 (67.5	218/429 (50.8)	267/457 (58.4)	1459/2582 (56.5)
Hb<8 g/dL, n/N (%)	45/424 (10.6)	54/424 (12.7)	46/424 (10.9)	55/424 (13.0)	42/429 (9.8)	36/457 (7.9)	278/2582 (10.8)
Birth weight in grams							
Mean (SD)	3130 (519)	3151 (499)	3183 (562)	3089 (518)	3022(516)	3042(445)	3094 (512)
LBW, n/N (%)	28/407 (6.9)	23/355 (6.5)	27/351 (7.7)	29/398 (7.3)	58/552 (10.5)	40/434 (9.2)	205/2497 (8.2)
Premature, n/N (%)	7/403 (1.7)	9/350 (2.6)	10/349 (2.9)	8/398 (2.0)	12/550 (2.2)	1/430 (0.2)	47/2480 (1.9)
SGA, n/N (%)	121/403 (30.0)	96/350 (27.4)	68/348 (19.5)	125/398 (31.4)	183/549 (33.3)	152/429 (35.4)	745/2477 (30.1)
Maternal parasitemia by microscopy							
Positive, n/N (%)	121/423 (28.6)	48/418 (11.5)	126/423 (29.8)	117/424 (27.6)	58/613 (9.5)	44/464 (9.5)	514/2765 (18.6)
GMPD, µl (95% CI)	1291 (877-1900)	1093 (587-2035)	1488 (1109-1997)	971 (690-1366)	1459 (805-2644)	1146 (599-2192)	1238 (1043-1471)
Maternal parasitemia by PCR		-	-	-	44/610 (7.2)	137/450 (30.4)	181/1060 (17.1)
Placental parasitemia by microscopy							
Positive, n/N (%)	88/423 (20.8)	49/418 (11.7)	126/407 (31.0)	99/424 (23.4)	51/602 (8.5)	31/463 (6.7)	444/2737 (16.2)
GMPD, µl (95% CI)	1600 (1060-2600)	1662 (938-2945)	1727 (1246-2396)	2274 (1587-3259)	2633 (1563-4436)	712 (352-1440)	1791 (1496-2144)
Placental parasitemia by PCR-		-	-	-	60/587 (10.2)	152/439 (34.6)	212/1026 (20.7)
Cord parasitemia by miscroscopy							
Positive, n/N (%)	0/411 (0)	0/414 (0)	0/403 (0)	0/420 (0)	0/600 (0)	6/461 (1.3)	6/2709 (0.22)
Cord parasitemia by PCR	-	-	-	-	16/497 (3.2)	80/368 (21.7)	96/865 (11.1)

Notes: N, Total sample size; n, number with events; SD, standard deviation; LBW, low birth weight; SGA, small for gestational age; GMPD, geometric mean of parasite density; µl, microliter; CI, confidence interval; C, centigrade; Hb, haemoglobin concentration; PCR, polymerase chain reaction (PCR for speciation was done only for two sites) .

* The high incidence of fever in San may reflect the high ambient temperature and the use of a defective thermometer. Hence the body temperature data was not used for any subsequent analysis in the different models.

6.3.2 Association between the number of SP doses received and birth outcomes

Clinical outcomes: Overall, use of 1 or more doses of IPTp-SP was associated with less LBW deliveries (Table 6.3), and severe anaemia (Table 6.4 & Figure 6.2), and higher mean haemoglobin concentrations, but not with a significantly higher mean birthweight (Table 6.5).

Infection status: IPTp was only associated with less maternal and placental malaria in primi and secundigravida, not in multigravida (Table 6.6 & Figure 6.2). SP dosing was not associated with preterm delivery or SGA (Table 6.3). In the 2 sites in 2009 that used detection PCR for malaria, two doses were also associated with a decrease in the risk of maternal (0.85 [0.56-1.00], p=0.049) and placental (0.62 [0.47-0.82], p=0.001) malaria infections among all gravida.

Table 6.3: Number of SP doses and prevalence risk of low birth weight and small for gestational age among women who delivered in the 5 districts

	LBW				Small for gestational age			
		No. with LBW (%)	*aPR (95% CI)	P-value		No. with SGA (%)	*aPR (95% CI)	P-value
All	N				N			
0 dose	714	87 (12.2)	1		705	220 (28.5)	1	
1 dose	882	62 (7.0)	0.63 (0.46-.86)	0.004	874	260 (35.4)	0.94 (0.81-1.09)	0.412
>=2 doses	896	56 (6.3)	0.57 (0.40-0.79)	0.001	893	264 (36.1)	0.96 (0.82-1.12)	0.574
G1-G2								
0 dose	294	50 (17.0)	1		290	111 (38.3)	1	
1 dose	358	40 (11.2)	0.68 (0.46-1.00)	0.050	354	134 (37.9)	1.02 (0.84-1.23)	0.860
>=2 doses	368	36 (9.8)	0.62 (0.41-0.95)	0.028	367	134 (36.5)	1.00 (0.81-1.23)	0.997
G3+								
0 dose	420	37 (8.8)	1		415	109 (26.3)	1	
1 dose	524	22 (4.2)	0.54 (0.31-0.94)	0.028	520	126 (24.2)	0.87 (0.69-1.10)	0.231
>=2 doses	528	20 (3.8)	0.45 (0.25-0.81)	0.008	526	130 (24.7)	0.91 (0.72-1.15)	0.434
ITN								
0 dose	454	51 (11.2)	1		450	139 (30.9)	1	
1 dose	592	43 (7.3)	0.67 (0.45-0.99)	0.047	586	167 (28.5)	0.87 (0.71-1.05)	0.143
>=2 doses	701	47 (6.7)	0.64 (0.42-0.96)	0.030	698	204 (29.2)	0.93 (0.77-1.13)	0.482
Non ITN								
0 dose	260	36 (13.9)	1		255	81 (31.8)	1	
1 dose	290	19 (6.6)	0.58 (0.34-0.98)	0.044	288	93 (32.3)	1.05 (0.83-1.33)	0.683
>=2 doses	195	9 (4.6)	0.41 (0.20-0.85)	0.016	195	60 (30.8)	0.96 (0.72-1.27)	0.758

Notes: LBW, low birth weight; APR, adjusted prevalence ratio; CI, confidence interval; N, sample size; SGA, small for gestational age; G1-G2, first or second pregnancy; G3+, three or more pregnancies ; ITN, insecticide treated net used last night.

*Adjusted for location (rural versus urban); age (≥18 versus <18 years), season of delivery, anaemia, site, and [gravidity (G1-G2 versus G3+) and ITN (users versus non-users) where applicable]

Table 6.4: Number of SP doses and prevalence risk of moderate to severe anaemia and severe anaemia among who delivered in the 5 districts

	Any anaemia (Hb < 11.0 g/dL)				Severe anaemia (Hb < 8.0 g/dL)			
		No. with anaemia (%)	*aPR (95% CI)	P-value		No. with severe anaemia (%)	*aPR (95% CI)	P-value
All	N				N			
0 dose	822	530 (64.5)	1		822	130 (15.8)	1	
1 dose	904	516 (57.1)	0.93(0.86-1.00)	0.059	904	80 (8.9)	0.69 (0.53-0.90)	0.007
>=2 doses	854	411 (48.1)	0.82 (0.74-0.89)	<0.001	854	68 (8.0)	0.69 (0.52-0.93)	0.013
G1-G2								
0 dose	333	233 (70.0)	1		333	62 (18.6)	1	
1 dose	371	226 (60.9)	0.92 (0.82-1.03)	0.138	371	40 (10.8)	0.68 (0.47-0.98)	0.037
>=2 doses	357	175 (49.0)	0.79 (0.69-0.91)	0.001	357	28 (7.8)	0.62 (0.40-0.97)	0.035
G3+								
0 dose	489	297 (60.7)	1		489	68 (13.9)	1	
1 dose	533	290 (54.4)	0.95 (0.85-1.06)	0.345	533	40 (7.5)	0.72 (0.49-1.07)	0.108
>=2 doses	497	236 (47.5)	0.84 (0.74-0.95)	0.006	497	40 (8.1)	0.75 (0.50-1.11)	0.153
ITN								
0 dose	510	306 (60.0)	1		510	74 (14.5)	1	
1 dose	593	345 (58.2)	0.98 (0.89-1.08)	0.701	593	44 (7.4)	0.58 (0.40-0.83)	0.003
>=2 doses	649	311 (47.9)	0.85 (0.76-0.95)	0.005	649	42 (6.5)	0.53 (0.36-0.77)	0.001
Non ITN								
0 dose	312	224 (71.8)	1		312	56 (18.0)		
1 dose	311	171 (55.0)	0.85 (0.75-0.97)	0.016	311	36 (11.6)	0.81 (0.54-1.21)	0.302
>=2 doses	205	100 (48.8)	0.77 (0.65-0.91)	0.002	205	26 (12.7)	0.95 (0.60-1.49)	0.815

Notes: aPR, adjusted prevalence ratio; CI, confidence interval; N, sample size; G1-G2, first or second pregnancy; G3+, three or more pregnancies ; ITN, insecticide treated net used last night.

*Adjusted for location (rural versus urban); age (≥18 versus <18 years), season of delivery, site, and [gravidity (G1-G2 versus G3+) and ITN (users versus non-users) where applicable].

Table 6.5: Number of SP doses and mean birth weight and haemoglobin among women who delivered in the 5 districts

	Mean birthweight		*Adjusted Mean difference, 95% CI			Mean haemoglobin		*Adjusted Mean difference, 95% CI		
All	N	Mean (SD)	grams	95% CI	P-value	N	Mean (SD)	g/dL	95% CI	P-value
0-dose	714	3067 (578)	ref			822	10.0 (2.2)	ref		
1-dose	882	3105 (504)	23.8	-27.8 to 73.3	0.377	904	10.6 (1.9)	0.46	0.27 to 0.65	<0.001
>=2 doses	896	3107 (463)	32.4	-18.6 to 83.4	0.213	854	10.9 (1.9)	0.70	0.51 to 0.89	<0.001
G1-G2										
0-dose	294	2940 (577)	ref			333	9.9 (2.2)	ref		
1-dose	358	2991 (540)	36.4	-43.6 to 116.4	0.372	371	10.6 (1.9)	0.56	0.27 to 0.86	<0.001
>=2 doses	368	3013 (452)	55.0	-27. to 136.6	0.186	357	10.9 (1.9)	0.95	0.64 to 1.25	<0.001
G3+										
0-dose	420	3156 (563)	ref			489	10.2 (2.2)	ref		
1-dose	524	3182 (462)	13.7	-51.8 to 79.2	0.682	533	10.8 (1.8)	0.38	0.13 to 0.63	0.003
>=2 doses	528	3172 (461)	19.3	-46.3 to 84.9	0.564	497	10.9 (1.9)	0.53	0.28 to 0.78	<0.001
ITN										
0-dose	545	3089 (581)	ref			510	10.2 (2.2)	ref		
1-dose	592	3101 (474)	9.4	-51.2 to 70.1	0.760	593	10.6 (1.7)	0.35	0.13 to 0.58	0.002
>=2 doses	701	3105 (458)	27.2	-32.1 to 86.5	0.368	649	10.9 (1.8)	0.63	0.40 to 0.85	<0.001
Non ITN										
0-dose	260	3030 (573)	ref			312	9.7 (2.2)	ref		
1-dose	290	3113 (560)	49.8	-44.1 to 143.7	0.298	311	10.5 (2.1)	0.61	0.26 to 0.96	0.001
>=2 doses	195	3116 (484)	45.9	-57.0 to 148.8	0.382	205	10.7 (2.1)	0.79	0.40 to 1.17	<0.001

Notes: SD, standard deviation; CI, confidence interval; N, sample size; G1-G2, first or second pregnancy; G3+, three or more pregnancies ; ITN, insecticide treated net used last night.

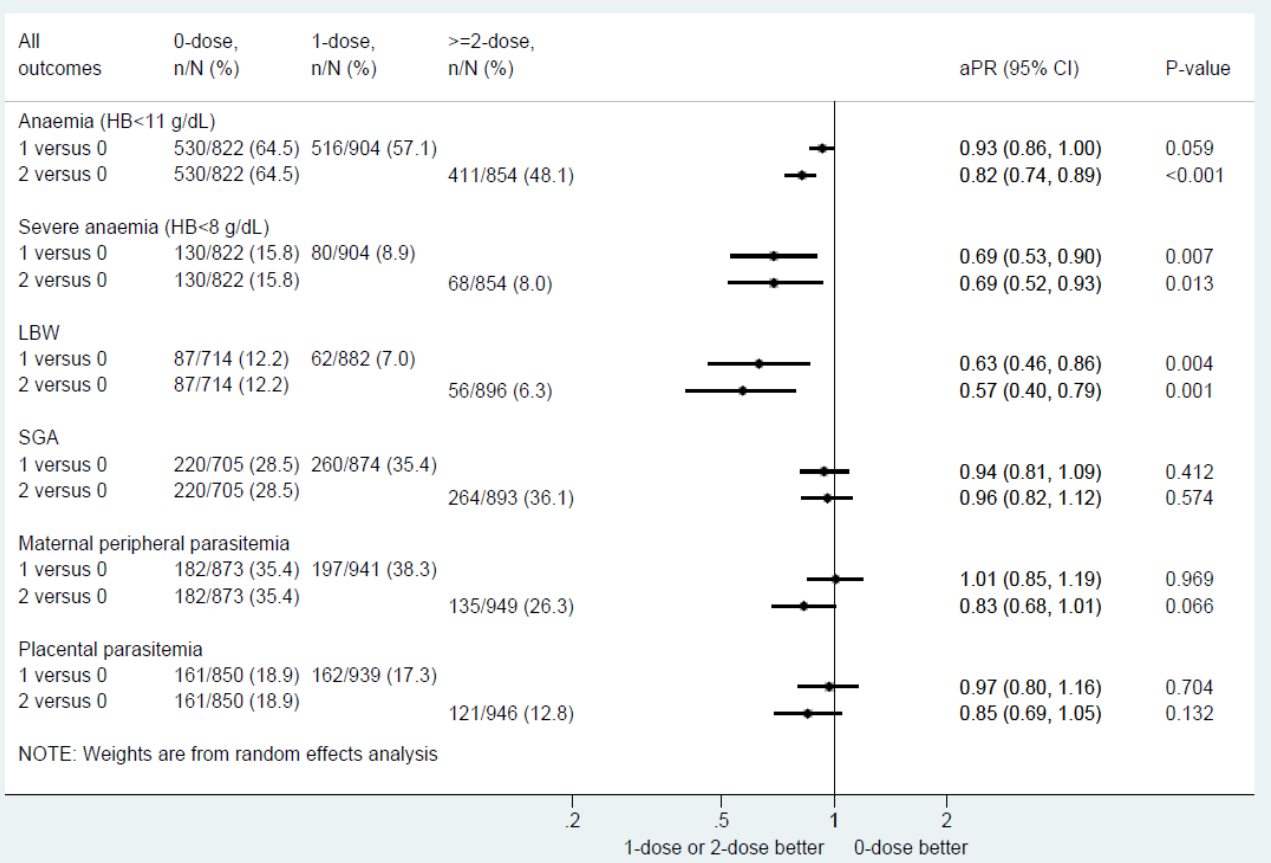
*Adjustment was done by location (rural versus urban); age (≥18 versus <18 years), season of delivery and [gravidity (G1-G2 versus G3+) and ITN (users versus non-users) where applicable], and anaemia (for birth weight) and without anaemia (for haemoglobin)

Table 6.6: Number of SP doses and prevalence risk of placental and maternal parasitemia among who delivered in the 5 districts

	Placental parasitemia				Maternal parasitemia			
		No. with infection (%)	*aPR (95% CI)	P-value		No. with infection (%)	*aPR (95% CI)	P-value
All	N				N			
0-dose	850	161 (18.9)	1		873	182 (35.4)	1	
1-dose	939	162 (17.3)	0.97(0.80-1.16)	0.704	941	197 (38.3)	1.01 (0.85-1.19)	0.969
>=2 doses	946	121 (12.8)	0.85 (0.69-1.05)	0.132	949	135 (26.3)	0.83 (0.68-1.01)	0.066
G1-G2								
0-dose	336	95 (28.3)	1		349	109 (31.2)	1	
1-dose	386	95 (24.6)	0.95 (0.76-1.19)	0.634	388	111 (28.6)	0.95 (0.78-1.17)	0.655
>=2 doses	398	67 (16.8)	0.77 (0.59-1.01)	0.062	400	77 (19.5)	0.77 (0.60-0.99)	0.042
G3+								
0-dose	514	66 (12.8)	1		524	73 (13.2)	1	
1-dose	553	67 (12.1)	1.00 (0.73-1.38)	0.994	553	86 (15.6)	1.12 (0.83-1.50)	0.452
>=2 doses	548	54 (9.9)	0.99 (0.70-1.40)	0.964	549	58 (10.6)	0.95 (0.68-1.33)	0.771
ITN								
0-dose	537	83 (15.5)	1		551	94 (17.1)	1	
1-dose	628	92 (14.7)	1.07 (0.82-1.40)	0.626	630	111 (17.6)	1.09 (0.85-1.38)	0.501
>=2 doses	741	74 (10.0)	0.83 (0.61-1.22)	0.227	744	80 (10.8)	0.78 (0.59-1.03)	0.081
Non ITN								
0 dose	313	78 (24.9)	1		322	88 (27.3)	1	
1 dose	311	70 (22.5)	0.89 (0.69-1.15)	0.375	311	86 (27.7)	0.94 (0.741.19)	0.619
>=2 doses	205	47 (22.9)	0.93 (0.69-1.25)	0.615	205	55 (26.8)	0.92 (0.70-1.21)	0.541

Notes: aPR, adjusted prevalence ratio; CI, confidence interval; N, sample size; G1-G2, first or second pregnancy ; G3+, third pregnancy or higher ; ITN, insecticide treated net used last night.
*Adjusted for location (rural versus urban); age (≥18 versus <18 years), season of delivery, site, and [gravidity (G1-G2 versus G3+) and ITN (users versus non-users) where applicable].

Figure 6.2: Number of SP doses and risk of adverse birth outcomes for women who delivered in the 5 districts



Notes: LBW, low birth weight; n, number of events; N, sample; CI, confidence interval; aPR, prevalence ratio adjusted for location (rural versus urban); age (≥18 versus <18 years), season of delivery, site, gravidity (G1-G2 versus G3+), ITN (users versus non-users), and anaemia (for LBW only); SGA, small for gestation age (defined as <10th percentile in birth weight for attained gestational age (Landis et al., 2009).

6.3.3 Molecular markers for SP resistance

The prevalence of the DHPS A437G and K540E mutation assessed by modelling (first 4 surveys) or pooled deep sequencing on samples from women attending ANC visit (pre-SP dosing, last 2 surveys), ranged from 15.2% to 44.8% for DHPS A437G and from 0 to 0.73% for *dhps* K540E mutation (Table 6.1). At delivery, the observed prevalence of triple *dhfr* (N51I, C59R, S108N) haplotype varied from 0.66% to 65.8% and that of quadruple *dhfr* (N51I, C59R, S108N)/*dhps* (A437G) haplotype from 22.2% to 42.1%; and no *dhps* K540E allele mutation was found (Table 6.1) .

6.4 Discussion

IPTp with SP was associated with significant and marked reductions in the risk of LBW (by 43%) and severe maternal anaemia (by 31%). The results were consistent across the 6 surveys and 5 sites with no evidence for heterogeneity across surveys (I^2=0%) and evident in all gravida groups (Figure 6. 3), including multigravida, and in ITN users and non-users. LBW due to preterm birth or intrauterine growth retardation is a strong predictor of infant health (Katz et al., 2013) thus the improvements in birth weight may have important health benefits in the first month to first year of life.

The risk reduction of LBW may not just reflect the effect of SP and may be confounded by other factors. For example, the number of ANC visits is likely to be correlated with the number of SP doses received (Van Eijk et al., 2004), and thus potentially more intensive or more complete antenatal care potentially resulting in improved birthweight. These include increased uptake of iron-folate tablet doses, tetanus toxoid, and treatment of potential infections. Other confounding factors such as a higher socio-economic status and education levels have also been found to be associated with high IPTp uptake (Van Eijk et al., 2011). Thus, because of the observational design used in our study, it may be possible that effect estimates overestimate the true effect of SP.

Our data are consistent with earlier findings from similar observational studies of IPTp with SP in west Africa including in Nigeria (Aziken et al., 2011), Burkina Faso (Sirima et al., 2006), Senegal (Olliaro et al., 2008), and Cote D'Ivoire (Vanga-Bosson et al., 2011), and Ghana (Tutu et al., 2011, Hommerich et al., 2007). Some of these earlier studies failed to show a statistically significant association with LBW, but that may be explained by the smaller

sample sizes in these earlier studies and other factors affecting the effect size such as high concomitant ITN usage or higher levels of SP resistance in the local parasite population.

Placental Malaria

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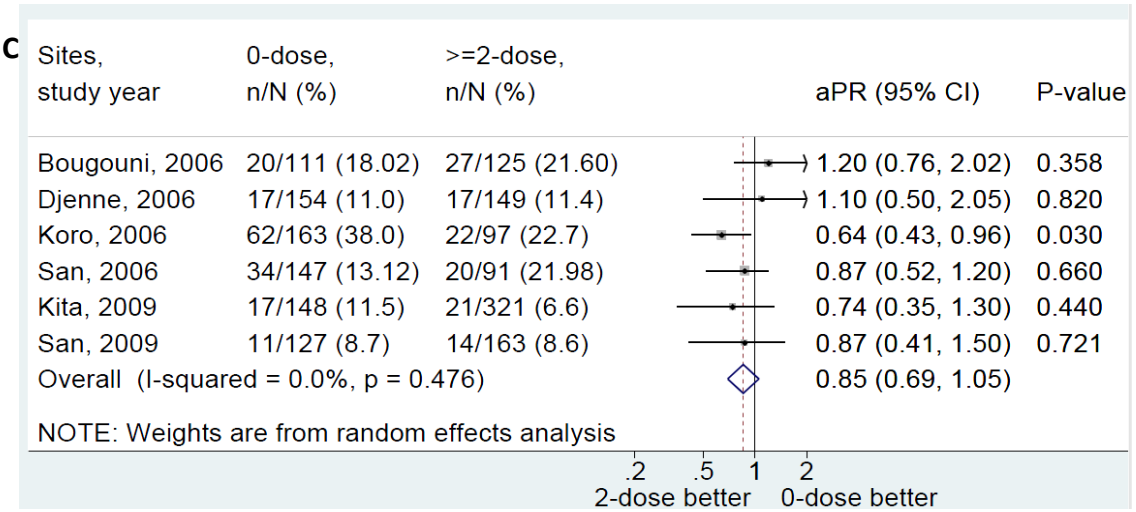
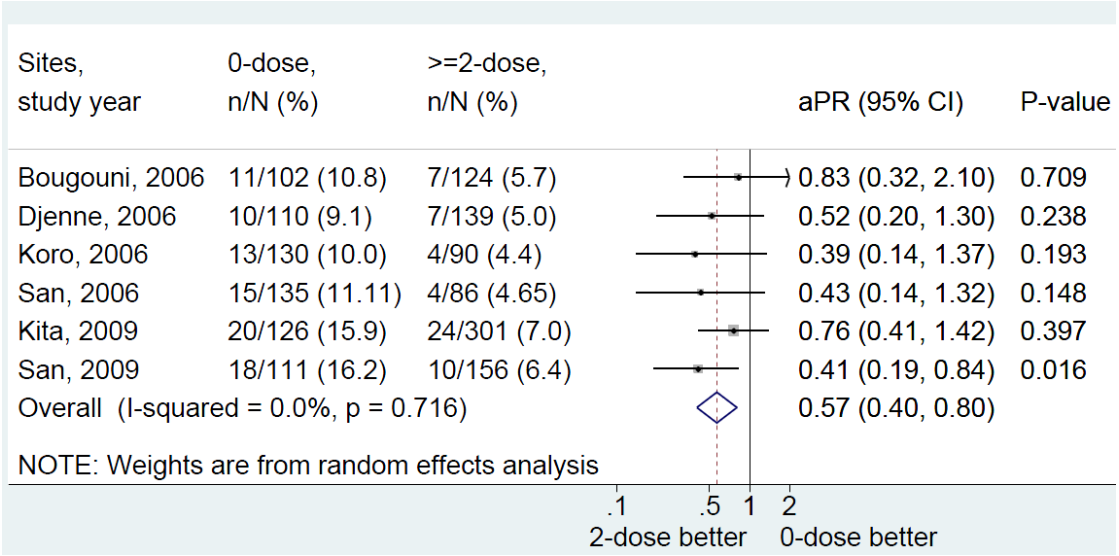


Low birth weight



Severe anaemia (HB<8 g/dL)

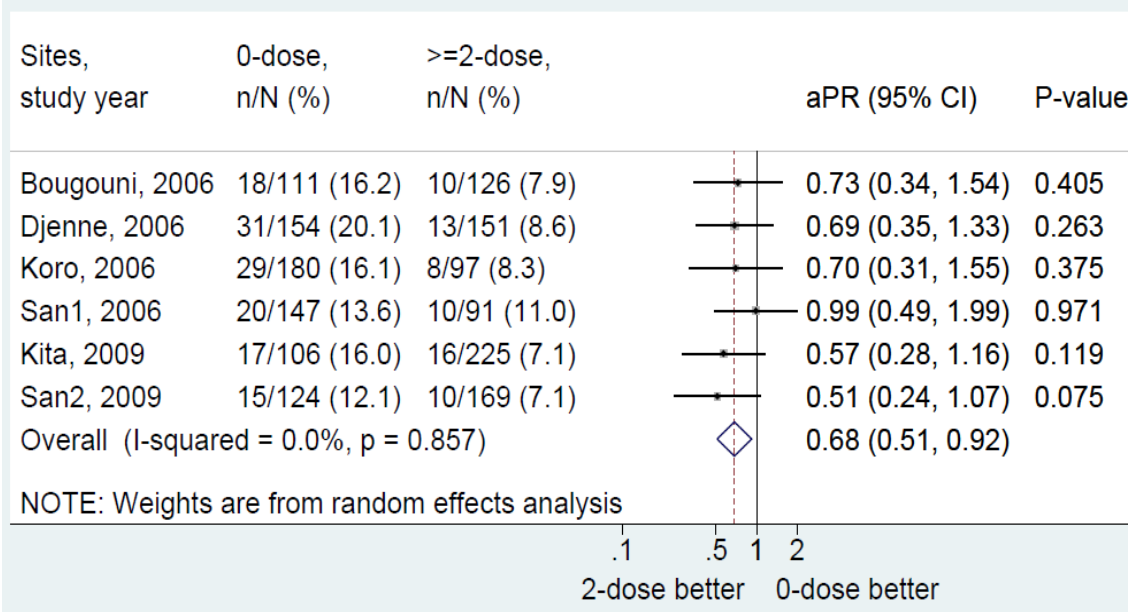


Figure 6. 3 : Associations between 2-dose IPTp use and risk of placental malaria, Low birth weight and severe anaemia in 6 surveys in 5 districts in Mali.

Note: n, number of events; N, sample; aPR, prevalence ratio obtained from random effects models adjusted for location (rural vs urban); age (≥18 vs <18 years), gravidity (G1-G2 vs G3+); ITN (users vs non-users); season of delivery, and anaemia (only for LBW); CI, confidence interval.
Severe anaemia was defined as haemoglobin level <8 g/dL.

By contrast to the beneficial association with LBW, the reduction of the risk of maternal and placental malaria among women with 2 doses of SP was smaller and not statistically significant. The most likely explanation, given the high sensitivity of parasites to SP observed in all 6 surveys and other studies in Mali (Diakite et al., 2011, Dicko et al., 2010, Kayentao et al., 2013, Tekete et al., 2009), is the possible re-infection of women after their last dose of SP as was suggested in earlier studies (Gies et al., 2009, Kayentao et al., 2005, Sirima et al., 2006). The median time between the last dose of SP and delivery was just over 10 weeks (72 days), and this was approximately 11 weeks (78 days) in women with placental malaria vs 71 days in women without evidence of infection at delivery (P=0.067) (Table 6.1) . The duration of post treatment prophylaxis which is likely to be an important determinant of the benefit of SP-IPTp, is approximately 6 to 8 weeks in areas with no or low SP resistance (White, 2005). Since over half of the women received their last dose over 10 weeks before delivery, this would have left women unprotected towards the end of their pregnancy after the protective drug levels have waned. This is especially a problem during the rainy season, when 85 % of deliveries in these surveys occurred. The high risk of reinfections argues in favour of the more frequent dosing regimen as was recently recommended by WHO (WHO, 2013). Three or more doses of IPTp-Sp with SP has been shown to markedly enhance the efficacy of IPTp in reducing the risk of low birth weight and severe maternal anaemia compared to the 2-dose regimen (Kayentao et al., 2013). It remains to be determined if this may also modulate the infant susceptibility to malaria (Mutabingwa et al., 2005, Schwarz et al., 2008, Harrington et al., 2013, Malhotra et al., 2009).

The risk reduction in severe maternal anaemia and the increase in mean haemoglobin levels achieved in women using IPTp-SP is also consistent with previous reports by other authors reporting a risk reduction in anaemia (Tutu E.O., 2010) or severe anaemia (Shulman, 1999) in women using 2 versus 0 doses of SP-IPTp or placebo. It may be possible that effect estimates in observational studies overestimate the true effect of SP, as women attending for antenatal care also receive iron-folate supplementation during ANC visits (not assessed in our study) and the amount of supplementation may be correlated with the number of ANC visits and thus the number of SP doses received, as suggested by other authors (Van Eijk et al., 2004).

By contrast to previous observations in clinical trials in Kenya (Njagi et al., 2003, Njagi, 2002), and the Gambia (Mbaye et al., 2006b), and observational studies in Malawi (Feng et al., 2010), we found that the 2-dose IPTp regimen was associated with the reduction in the risk of severe anaemia and LBW among both ITN non-users and users, although the effect appeared greatest among non-users. The possible explanation for the significant effects among women protected by ITN could be the very high transmission pressure during the surveys, most of which coincided, at least in part, with the transmission season. It is possible that under such condition single intervention is inadequate to provide complete protection. Other explanations include discrepancies between ITN ownership and consistent use during pregnancy (not assessed).

This observational study is not free of limitations. The study did not permit a critical assessment of effect modification by season of the association between IPTp-SP use on birth outcomes because the surveys were timed to coincide with the rainy season and 85.2% of observed deliveries occurred during the transmission season. Malaria transmission is highly seasonal in Mali and the malaria attributable fraction towards LBW will be lower outside of the transmission seasons. It is thus likely that our study estimates obtained during the transmission seasons, overestimate the average impact that can be expected throughout the year.

The analysis was in part designed to assess the impact of SP resistance on IPTp effectiveness. Although data on the frequency of SP resistance was available from all surveys at the time of delivery, this is not the best time to assess the parasite population level of resistance, as parasites present at delivery may have been selected under drug pressure from SP use during pregnancy. Nevertheless, the molecular data available at delivery suggested that parasites remain highly sensitive to SP in Mali where the *dhps* K540E mutation is largely absent and the prevalence of the *dhps* A437G mutation was <50%. This is also evident from the findings of the 42-day *in vivo* efficacy studies conducted in 2009 in two of the 5 sites, which showed near complete radical cure after a single dose of SP in asymptomatic women who were parasitaemic when they received their first dose of SP (Coulibaly et al., 2014) . The lack of clinically relevant variation in resistance between the sites prevented a meaningful analysis of the correlation between

the prevalence of SP molecular markers such as *dhps* A437G and the effectiveness of IPTp.

Of note was the high proportion of women who received only one dose (34%) or no IPTp-SP (32%) despite the support from multiple donors provided to these specific sites. Even in the survey conducted in 2012, 8 years after the introduction of IPTp, the 20% coverage of >=2-dose failed to reach the RMB targets of 60% by 2005 and 80% by 2010 by a wide margin (WHO, 2000a, van Eijk et al., 2013). Although reasons for the low coverage are not fully understood, recent studies in Mali identified many missed opportunities due to factors related to poor ANC attendance or living outside the health facilities catchment area, but also because the delivery of IPTp was ineffective due to complex policy guidelines, lack of guidance on how to implement the guidelines, and the institutionalising of practices that undermine the national guidelines (Mali, Mars 2011). Although there were a number of broader health systems issues which were not specific to IPTp or antenatal care, other factor which were potentially actionable, accounted for the majority of the substantial loss in system effectiveness observed. These include misconceptions about the optimal timing of IPTp and specifically the upper limit of the gestational age at which it could be given, the required interval between doses and concerns about giving IPTp-SP on an empty stomach (Webster et al., 2013b). These could be addressed in the shorter-term whilst waiting for broader health systems issues need to be addressed.

6.5 Conclusion

Our study confirms that the parasite population in Mali remains highly sensitive to SP and that IPTp-SP is very effective in reducing the adverse consequences of malaria in pregnancy among women who receive at least 2 doses of SP. However the uptake of the 2-dose regimen is disappointingly low due to ineffective processes in the delivery of IPTp. We also observed a very high risk of placental malaria in the 2-dose recipients, presumably reflecting the high risk of reinfection between the last dose of SP and delivery. WHO's new IPTp policy update aims to address many of these intervention-specific factors by simplifying the guidelines and improving messaging. The increase in the number of doses of SP from 2 doses to a dose at each scheduled antenatal visit in the 2nd and 3rd trimester at least one month apart ensures better alignment with WHO's

focused antenatal care schedule (WHO, 2013, van Eijk et al., 2013). This combined with the superior efficacy of this new regimen observed in trials (Kayentao et al., 2013) , including in Mali (Diakite et al., 2011), strongly suggest that these new WHO guidelines should be implemented as soon as possible with the aim to improving the coverage and effectiveness of this promising, yet underutilised intervention.

Chapter 7: General discussion and conclusion

7. Chapter 7-General discussion and conclusion

Since the first descriptions of the association between malaria and pregnancy in the early 20th century (Desai et al., 2007), malaria continues to burden pregnant women and her offspring. This is despite tremendous investments made recently in the fight against malaria through the RBM led initiative to control and eliminate the disease. In pregnant women living in sub-Saharan Africa, *P. falciparum* malaria is still one of the biggest public health problems and facing several major challenges related to its control requiring continued attention to the diagnosis, treatment and prevention of malaria in pregnancy. This thesis provides further insights into the epidemiology and burden of malaria in pregnancy in an area with intense, highly seasonal malaria, typical for the Sahel region and suggests improvements to the IPTp regimen using SP, the main drug-based prevention strategy in pregnant women, and evaluated the relationship between SP resistance and the effectiveness of IPTp in West Africa. This discussion chapter is structured around 4 themes based on the main results of this thesis.

7.1 Burden of malaria in pregnancy in Mali

The present thesis benefits from a wealth of data collected in 11 surveys conducted from January 2005 to December 2010 on the burden of malaria in pregnancy in different regions and transmission settings of Mali. It provides for the first time a national estimate analysis of pregnancy associated malaria (PAM) and emphasizes the potential benefit of the main two pronged approach recommended by WHO for malaria prevention during pregnancy in sub-Saharan Africa (WHO/AFRO, 2004). In that, the work addressed in chapter 2 of this thesis can be very informative as baseline information for future evaluation of PAM and its control strategies at regional or national level. It showed that malaria is highly seasonal, but that the intensity of its transmission suggested a marked regional variability with some unexpected changes in the transmission landscape unlike previously described in 1991 (Doumbo O., 1991). This may indicate the need of revising the county wide malaria transmission maps to reflect the recent ecological changes that have occurred in Mali with the introduction of large-scale irrigation projects that may have become the main drivers of transmission in the dry season in some areas. For example in Timbuktu, an area that was classified *a priori* as low transmission based on

the existing classification used in Mali, our observations suggested a prevalence of placental malaria infection of 13.3% in dry season which was almost 2 fold higher than that reported for Kita with 7.3%, an area classified *a priori* as high transmission. The prevalence of *P. falciparum* malaria of 13.3% in Timbuktu was a surprise as the survey took place towards the end of the dry season. The true prevalence at delivery and corresponding impact on birth outcomes may even be considerably higher if more sensitive diagnostic methods such as PCR and placental histology were used (Fried et al., 2012, Taylor et al., 2010). Although PCR was not available for the Timbuktu surveys, diagnostic PCR data was available in the last two surveys conducted in 2009 in San and Kita, described in chapter 6. This suggested that the prevalence of *P. falciparum* infection detected by PCR was 3 times (maternal), ≥ 5 times (placental), and 50 times (cord blood) higher than the estimates obtained by microscopy (Table 6. 2). Other contributing factors to the difference observed between the traditional transmission strata and the prevalence data observed in pregnant women at delivery may include the persistence of parasitaemia for many months into the dry season and improvements in the coverage of malaria control strategies as observed in Kita (Table 2.8).

In Timbuktu, despite the unexpected high prevalence of both maternal and placental malaria, no *P. vivax* infection was found, yet previous studies reported the presence of *P. vivax* in this site (Bernabeu et al., 2012) and other sites of northern Mali with similar characteristics (Koita et al., 2012, Doumbo O., 1991). However, the absence of *P. vivax* in our study does not preclude its presence in this ethnically and genetically diverse regions, where 34% of the population are Tuaregs (Timbuktu-Region), sometimes referred to locally as 'brown' people. They may have a Duffy-positive blood group antigen, which also serves as a receptor (the portal of entry) for *P. vivax* to invade red blood cells (Bernabeu et al., 2012). It is also possible that the Tuaregs, who traditionally have a nomadic pastoralist lifestyle, are relatively underrepresented among the antenatal clinic population. The presence of *P. vivax* in previous studies (Koita et al., 2012, Doumbo O., 1991, Bernabeu et al., 2012) may also be suggestive of lack of power of the diagnostic method used (microscopy) in our study and the difficulty of accurate species differentiation at the low asexual and sexual stage parasite densities that are typical for semi-immune pregnant women.

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In accordance with the literature (chapter 1), this thesis demonstrated that first and second pregnancies are most burdened by malaria and its impact, in that higher prevalence of placental malaria, LBW, and maternal anaemia were observed in that group as opposed to more than two pregnancies (chapter 2). Although malaria is a major contributing factor to LBW (Desai et al., 2007), our ecological analysis showed similar prevalence of LBW across transmission settings. Nevertheless, in chapter 2 and chapter 6 it is shown that placental malaria was associated with higher risk of LBW, SGA, and the composite LBW or SGA or PTD and IPTp use was associated with a lower prevalence of LBW and maternal anaemia for which malaria is also a considerable contributor (Rogerson et al., 2007a, Katz et al., 2013, Desai et al., 2007).

Although malaria is a contributor to LBW, our ecological analysis did not find any link between LBW and malaria transmission intensity. Thus, other factors in malaria endemic areas not assessed in our study may play an important role for the occurrence of LBW (Huang et al., 2007, Guyatt and Snow, 2004).

A relatively low uptake of IPTp was observed (chapter 2). This suggests significant missed opportunities to further reduce the impact of malaria on new-born health. Our study suggested that increasing IPTp coverage to the audacious RBM target of universal coverage could result in a significant decrease of births with placental malaria, LBW, and anaemia among first and second pregnancies.

Despite several limitations described in chapter 2, this is the first comprehensive study of the burden of PAM suggesting that malaria in pregnancy is a major public health problem in all endemic areas and suggest the potential for further reductions in the burden throughout Mali by upscaling IPTp and ITN coverage in pregnancy. For a better description of the true burden of PAM at the national level in this country with such varied transmission strata, prospective surveys should reflect seasonality, transmission intensity, and ideally use more sensitive diagnostic tests for malaria detection. Such studies are required for building malaria control strategies and to rationalize the distribution of scarce resources.

7.2 Increasing the dosing frequency of intermittent preventive therapy with sulfadoxine-pyrimethamine in Africa

The trial conducted in Mali in 1998-2001 suggested that IPT-SP was superior to chemoprophylaxis with chloroquine. However, the prevalence of placental malaria was higher than expected among women taking two doses of SP (Kayentao et al., 2005). This was confirmed later by a more recent study in another part of the country (Diakite et al., 2011) (chapter 3). As parasites are very sensitive to SP (chapter 5) in Mali, this high rate of placental malaria was more likely to reflect re-infection during the last 6-10 weeks gestation, an important period for foetal growth and weigh gain. This was further supported by our results of the observational studies described in chapter 6 showing that the median time from last dose of SP to delivery was around 10 to 11 weeks.

Thus, more frequent doses of IPTp were suggested to prevent reinfection towards term. Testing this hypothesis, the trial described in Chapter 3 showed that three doses of IPTp with SP was considerably more effective in reducing maternal and placental malaria, low birth weight and premature delivery than the 2-dose regimen (chapter 3). Furthermore, the extra dose of SP was well tolerated (Diakite et al., 2011). This was the first trial that compares the 2-dose regimen to more frequent dosing schedules in west-Africa, it provided the evidence that more than 2-dose of SP would be more appropriate for the control of malaria in pregnancy and its sequelae in Mali and in the Sahel countries in sub-Saharan west Africa, most of which currently have low levels of SP resistance (Naidoo and Roper, 2013).

The study was followed by a meta-analysis that combined the result of this trial with evidence from 6 other clinical trials (chapter 4); including 1 similar trial comparing 3-dose versus 2-dose in a low SP resistance area in Burkina Faso, West Africa and 5 trials comparing monthly versus the 2-dose regimen in eastern and southern Africa in areas with high SP resistance. The pooled analysis confirmed the superiority of 3 or more doses over the 2-dose regimen in the reduction of placental malaria and low birth weight in both areas with low and moderate to high SP resistance (Kayentao et al., 2013). Among HIV infected first and second pregnancies, a previous meta-analysis of 3 trials suggested significant benefit of additional doses of sulfadoxine-pyrimethamine over the 2-dose regimen regardless of the degree of sulfadoxine-pyrimethamine resistance, but was

unpowered to conclude similar benefit among HIV negative pregnant women (Ter Kuile et al., 2007). Thus, our new meta-analysis of 7 trials of which 6 enrolled HIV negative pregnant women allowed the evidence of this benefit to emerge and showed that this occurs regardless of HIV status and the degree of SP resistance (Kayentao et al., 2013). The meta-analysis also showed that increasing the dosing frequency is not associated with an increase in SP related serious adverse events. This data provided support for the new WHO recommendation that intermittent preventive therapy during pregnancy with sulfadoxine-pyrimethamine should be provided at each scheduled focused antenatal-care visit in the second and third trimesters in all settings in which intermittent preventive therapy during pregnancy with sulfadoxine-pyrimethamine is recommended (WHO, 2013).

Countries with 2-dose regimen are now revising their policy for IPTp-SP frequent dosing. However, there is a need for research to be focused on how best to implement the updated WHO guidelines for IPT-SP in pregnant women, especially their integration with focused antenatal care, as recent reviews of surveys from 2009-2011 show that ¾ of sub-Saharan-African countries have struggled to attain the original Abuja target of 60% by 2005, a *fortiori* the 80% by 2010 (van Eijk et al., 2013). Nonetheless, the implementation of the updated policy may benefit from the lesson learned from a recent series of studies conducted to identify the successes and impediments to high coverage of the IPTp-SP 2-dose strategy (Webster et al., 2013b, Webster et al., 2013a, van Eijk et al., 2013). This showed that the coverage of IPTp was lower in uneducated, poorer, and rural women (Hill et al., 2013, van Eijk et al., 2013, Steketee and Eisele, 2009) and higher in educated and the wealthier, who usually live in urban areas where the need of IPTp is less crucial because of lower malaria endemicity (van Eijk et al., 2013). The programmatic deployment of the new strategy should take these findings of inequalities among IPTp-recipients into account to ensure that the new strategy genuinely reaches women most in need. As the majority of sub-Saharan African countries struggle to support their own implementation program; and funding from international donors is frequently subject to shortfall, there is an urgent need for building a sustainable strategy for permanent domestic funding of implementation and surveillance programs for malaria control in

pregnant women. This is required to ensure high use and impact of the revised policy of IPTp and other malaria control strategies, in the era toward malaria elimination.

7.3 Sulfadoxine-pyrimethamine resistance and IPTp-SP effectiveness on birth parameters

With the anticipated further increase in use of SP for IPTp and seasonal malaria chemoprevention, the continued monitoring of SP resistance and the effectiveness of IPTp during pregnancy is essential. Parasite populations in western Africa seems to be mostly sensitive to SP (Naidoo and Roper, 2013, Flegg et al., 2013), and IPTp-SP has proven to be highly effective and efficacious in clinical trials and observational studies (Valea et al., 2010, Sirima et al., 2006, Kayentao et al., 2005, Diakite et al., 2011). However, spread of SP resistance from eastern and southern Africa, or de –novo development of high-level SP resistance may occur and monitoring of the effectiveness of IPTp with SP is essential.

Previously, monitoring of SP resistance was based on results from in-vivo studies among symptomatic children under 5 years old with uncomplicated malaria. However this does not correlate with the response to treatment with SP in pregnant women with malaria (Kalanda et al., 2006, Tagbor et al., 2007). Thus, designing a study for the better understanding of the efficacy of IPTp-SP in pregnant women was essential. In chapters 5 and 6 the results are describing studies aiming at obtaining a better understanding of the impact of SP resistance on the effectiveness of IPTp in clearing existing parasites and the duration of post-treatment prophylaxis to prevent new infections (Chapter 5), as well as on birth parameters in western Africa (Chapter 6).

The 42-day *in vivo* efficacy study reported in Chapter 5 of this thesis was conducted in Mali and Burkina-Faso, two West African countries where malaria transmission is endemic but seasonal. This was among the first studies to examine the 42-day *in vivo* response of IPTp-SP in asymptomatic women in West Africa, and showed that SP remains very effective at clearing existing infections and improving haemoglobin concentration when provided as IPTp to parasitaemic asymptomatic pregnant women. The *dhps* K540E mutation, which is a proxy for the quintuple haplotype conferring mid-level resistance to SP, was present in only one of the two sites of Mali and at very low frequency (0.73%,

95% CI 0.58-0.87). The mutation at *dhfr* codon 164L and *dhps* codon 581G conferring very high-level resistance to SP were absent. Our observations provide an important contribution to the understanding of the predictive value of the frequency of population estimates of the different *dhfr* and *dhps* mutations on the efficacy of SP in clearing malaria infection among asymptomatic pregnant women, especially when our results are compared against day 42 failure rates in areas with higher resistance. For example, the *dhfr* triple mutation (Ile51+Arg59+Asn108) is known to confer intense pyrimethamine resistance *in vitro* (Gregson and Plowe, 2005) and is associated with an approximate 1,000-fold reduction in pyrimethamine susceptibility (White, 2005) and was present in almost 50% of the parasite population in Burkina Faso, yet only 1.4% of the treatments recrudesced by day 42. This contrasts with previous findings in children with acute malaria, among whom the *dhfr* triple mutation was associated with an increased risk of SP treatment failure (Picot et al., 2009, Mockenhaupt et al., 2005, Kublin et al., 2002).

IPTp-SP targets are birth weight and maternal anaemia, and low birth weight or anaemia have been associated separately or in concert to lead to neonatal and infant mortality (Desai et al., 2007). Monitoring both birth weight and anaemia are therefore essential markers for assessing IPTp-SP effectiveness.

The results described in Chapter 6 suggest a strong beneficial association between the prevalence of LBW, and moderate-severe anaemia with the number of SP doses received.

The study failed to show a correlation between the relative effectiveness and the level of SP resistance in the population as measured by the prevalence of different molecular markers. This is possibly due to the modest prevalence of both triple *dhfr* (N51I, C59R, S108N) and quadruple *dhfr/dhps* (Triple *dhfr*+ *dhps* A437G) mutants which were 50% and 35%, respectively, and near absence of the *dhps* K540E mutation which is a good proxy for the quintuple haplotype. The effectiveness on delivery parameters and absence of a correlation with resistance markers is consistent with the good in-vivo response observed in chapter 5, suggesting that SP resistance is not yet a problem in Mali or this northern part of Burkina Faso.

Results generated in chapter 5 and 6 had contributed significantly to the first multicountry effort to link population level of SP resistance and efficacy and effectiveness

of IPTp. Using meta-analysis approach, our findings in chapter 6 were combined with similar studies conducted in 4 countries of West Africa. A total of 11 studies observational studies concluded a significant pooled risk reduction of LBW in women with 2 doses versus those not receiving SP (Figure 9.1 in annex). This analysis also showed for the first time a decrease in birth weight with an increase in DHPS 437 mutations level in West Africa (Figure 9.2 & Figure 9 3 in annex).

Another important finding is the high proportion of women who received only one (34%) or even zero doses (32%) of IPTp-SP. Despite the support from multiple donors, in 2010 the overall and specific site coverage of >=2-dose had never reached the RMB targets of 60% by 2005 and 80% by 2010 (RBM, 2008, RBM, 2011, van Eijk et al., 2013). This is not unique to Mali and this observation was similar to that reported earlier for sub-Saharan Africa as a whole (van Eijk et al., 2013). This maybe a forewarning of difficulty days ahead for the updated IPTp policy (WHO, 2013). Thus, there is clear suggestion of additional efforts from program managers of African countries to achieve primarily their own target and secondly the above mentioned international target which was more recently updated to universal coverage (100%) (RBM, 2011).

Results gathered from chapter 5 and 6 can be added to the mass body of data collected in other countries of sub-Sahara Africa to generate a practical standard protocol for monitoring the impact of SP resistance on IPTp-SP effectiveness in pregnant women (http://www.mip-consortium.org/ P: https://portal.mip-consortium.org) and to help define thresholds of SP resistance when countries should consider switching to alternative drugs and strategies.

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All the chapters from this thesis provided useful national and regional information of public health interest. However, unlike chapter 3 and chapter 4 which were randomized controlled trials, our data generated in observational studies discussed in chapter 2, chapter 5 and chapter 6 are susceptible to bias as in any observational study. Although multi-variate analysis was used to adjust for known confounders where available, residual confounding cannot be excluded and the results need to be interpreted with caution as systematic bias can distort the estimated associations between a risk factor and outcomes.

However, depending on the context, observational studies might help drive policy, especially because the results at delivery (most prone to bias) were supported by high levels of observed efficacy of SP in the in-vivo study in Mali and Burkina, and by the absence or very low population prevalence of the *dhps* 540 mutations (chapter 5) and a <50% prevalence of the *dhps* A437G mutation (chapter 6). This suggested that SP resistance is still low to modest in west Africa, and that IPTp-SP potentially has a long life ahead in Mali and Burkina, unlike in east and southern Africa, where the observational studies at delivery showed a lack of impact of IPTp-SP on birthweight in areas with high frequency of the *dhps* 540 mutation and where the additional mutation *dhps* 581 is present at > 10% (described as areas with super-SP resistances). Clinical trials are nearing completion to determine whether ISTP or IPTp with AL or DHA-PPQ may provide suitable alternative in these high resistance settings. A randomized controlled trial conducted in Ghana demonstrated that ISTp was not inferior to IPTp-SP in reducing the risk of LBW and third trimester anaemia (Tagbor et al., 2010). This was confirmed recently by data from a multicountry non-inferiority randomized controlled trial enrolling more than 5,000 primi and second gravida (Tagbor et al, manuscript in preparation). Thus, for some location such as in northern Tanzania, switching to alternatives such as ISTp may be warranted even before definitive trial results are available, because clinical trials results take time, yet in the interim time pregnant women and their offspring are suffering.

7.4 Ongoing and future research

7.4.1 Burden of malaria in pregancy

In Mali, a comprehensive diversity of malaria transmission entities and parasites species was first described in 1991 (Doumbo O., 1991). In community surveys conducted in 2010 (PNLP-Mali, 2010) and 2012 (DHS, 2012-2013), the prevalence of parasitemia reported in children aged 6-59 months had shown a significant increase from 2010 to 2012 in some regions of the country indicating clearly that malaria is not declining in Mali, and is even increasing in parts of the country. This was contrary to a recent report on the global decline of malaria incidence, including in Africa (Kweka E.; Mazigo, 2013). Our evaluation (of which the last was conducted in 2010) of the burden of malaria during pregnancy carried out during a 5-year period in 9 different sites may not reflect the current situation because of increasing use of malaria control strategies, changes due to global warming or other ecological changes and increased human population activities. This may partially explain the case of Timbuktu, with unexpected high prevalence of placental malaria (chapter 2) and that of Kita with low prevalence. In addition, our study did not find any *P. vivax* as opposed to previous studies carried out in the region (Koita et al., 2012, Doumbo O., 1991, Bernabeu et al., 2012). Thus, an updated assessment of PAM data is required at national level, especially in northern Mali where malaria transmission is traditionally low or epidemic. Prospective burden surveys should aim to capture data throughout the year, ideally using sensitive diagnostic tests to detect malaria infection. Such a survey should include coverage estimates of interventions and assessment of molecular markers of SP resistance, for example by adding a malaria in pregnancy module to National Malaria Indicator Surveys or DHS surveys.

7.4.2 Implementation of the updated WHO guidelines on IPTp-SP

Despite one to two decades of implementation of IPTp-SP within national programmes in sub-Saharan African countries, the coverage of this proven intervention for protecting pregnant women is still low (van Eijk et al., 2013). Reasons have been extensively reported among which weak health systems, a lack of reasonably accurate monitoring data, and inadequate use of data for managing programmes at local level (Webster et al., 2013b, Webster et al., 2013a, Hill and Kazembe, 2006, Hill et al., 2013). Thus, although much evidence exists on the efficacy of IPTp, there is little evidence on how best to

deploy it at programmatic conditions. Based on new findings from a meta-analysis (chapter 4), new WHO guidelines now recommend one IPTp-SP dose at each scheduled ANC visit from the second trimester (WHO, 2013). If no dedicated action is taken, this recommendation would face the same coverage issues as before.

There is therefore an urgent need for research to be focused on how best to implement the updated WHO guidelines for IPT-SP in pregnant women, and especially their integration with focused antenatal care through which IPTp-SP is delivered. This requires a continued identification and quantification of barriers to IPTp-SP high coverage at district and facility levels through operational research and good monitoring methods as suggested earlier (Webster et al., 2013b). Moreover, additional training may be needed as well as revision of data collection tools and malaria in pregnancy indicators.

7.4.3 Alternative to SP for IPTp

Although results for western Africa look reassuring, a worrying lack of effectiveness on birth parameters has been observed in parts of eastern and southern Africa (Kalilani et al., 2011, Harrington et al., 2011). Presently, SP is the only antimalarial recommended for IPTp (WHO/AFRO, 2004). It has ideal properties for intermittent use; it can be given as a single stated dose as directly observed therapy in the clinics, it is cheap, widely available, has good reputation among health care workers and end users and is well tolerated and safe in the second and third trimesters of pregnancy. With all these qualities, it is a big challenge to replace this valuable drug for IPTp. Potential alternative drugs in the pipeline include 1) Mefloquine (effective but poor safety and tolerability profile (Briand et al., 2009, Chico and Chandramohan, 2011b, Nosten et al., 2007)), 2) chloroquine-azithromycine combination (good safety but weak tolerability profile of high-dose azithromycin), 3) dihydroartemisinin-piperaquine (good efficacy profile, but more data needed on its safety and tolerability profile in pregnancy (Davis et al., 2010)). Investigations of these drugs for IPTp are almost completed, most of them under the umbrella of the malaria in pregnancy consortium (http://www.mip-consortium.org/ P: https://portal.mip-consortium.org). In addition, under the sponsorship of the National Institute of Allergy and Infectious Diseases (NIAID), a randomized, controlled trial is being conducted in Malawi on chloroquine (CQ) as chemoprophylaxis versus IPTp (SP or CQ) to prevent malaria in pregnancy (NCT01443130, ClinicalTrials.gov). Even though this trial

may result in CQ being considered for IPTp, the previous non-compliance with CQ prophylaxis (Denoeud et al., 2007) and pruritus induced by CQ (Onyeji and Ogunbona, 2001) might be important programmatic barriers towards the reintroduction of CQ in malaria prevention during pregnancy.

7.4.4 Intermittent screening and treatment strategy

The concept of this strategy is to provide scheduled RDT-based screening as part of focused antenatal care (FANC) and treatment of those with positive results with an effective and long acting ACT during the second and third trimesters. Screening periods must be at least one month apart (4-8 weeks) with the aim of treating existing infection and providing post-treatment prophylaxis for up to six weeks (White, 2005). Recent randomized controlled trials conducted in Ghana (Tagbor et al., 2010) and other western African countries (http://www.mip-consortium.org/ P: https://portal.mip-consortium.org) suggested the non –inferiority of this strategy to the standard IPTp-SP on birth parameters (Tagbor et al, unpublished). Although another advantage of this strategy is to reduce the exposure to unnecessary drug pressure in non-infected women, especially in areas with low malaria endemicity or under seasonal transmission, its success as a viable alternative to IPTp-SP relies also on the use of RDTs and their sensitivity to detect low-level, chronic placental infections. A combined HRP2 and pLDH based antigen, prequalified by WHO should be recommended (WHO-FIND, 2009), as it has the advantage to increase the test sensitivity to detect *P. falciparum* infections and other pan-species. Other important issues related to this strategy are: 1) the non-adherence of health care providers, 2) lack of procurement and good storage of RDT kits, 3) non-availability of adequate drug for treatments.

In the absence of an alternative drugs to replace SP, this ISTp strategy can be a good alternative in areas with high SP resistance, especially in eastern and southern Africa, and also in areas with significant decline of malaria incidence such as Zanzibar (Bhattarai et al., 2007) and the Gambia (Ceesay et al., 2012, Ceesay et al., 2010). In the mid or long term, it can be an attractive strategy in other countries of Africa in the context of continuing global decline of malaria incidence when countries are moving towards pre-elimination (van Eijk et al., 2013). However, whether this strategy is operationally

feasible and will be a cost effective alternative to IPTp-SP where SP resistance is high, or in areas where malaria is declining remains to be determined.

Furthermore a better understanding of the clinical implications is required of low densities infections that are potentially missed by microscopy or RDTs during the screening for malaria in pregnant women (Fried et al., 2012). Research is also needed to improve diagnostic test options that have greater sensitivity (without affecting specificity) and can be used at point of care. Nonetheless, because of the current lack of a suitable replacement drug to SP for IPTp, and based on the current evidence of the efficacy of ISTp despite the limited sensitivity of the existing RDTs, ISTp is currently the most attractive and promising strategy that could potentially replace IPTp-SP in areas of high SP resistance or low malaria transmission intensity. Further studies of its feasibility and analysis of the cost effectiveness of ISTp are therefore urgently needed.

7.4.5 Monitoring of SP resistance

In the presence of the alarming levels of high-grade SP resistance in parts of eastern and southern Africa and evidence for the decreased effectiveness of SP associated with an increased prevalence of DHPS A437G in West Africa, even in the absence of the additional DHPS K540E mutation (Figure 9. 2 & Figure 9. 3 in annex) there is a clear need to continue monitoring of SP resistance and to assess its impact on birth parameters. This is especially important with the recent introduction of seasonal malaria chemoprevention with AQ-SP combination in the Sahel countries. In countries with low levels of the DHPS K540E mutation, DHPS A437G can be used as a good molecular marker of SP resistance in the population to correlate with clinical markers of IPTp-SP effectiveness on birth parameters. A simple standardized protocol is being developed by the malaria in pregnancy consortium in collaboration with WHO to direct the monitoring of IPTp-SP in Arica.

8. References

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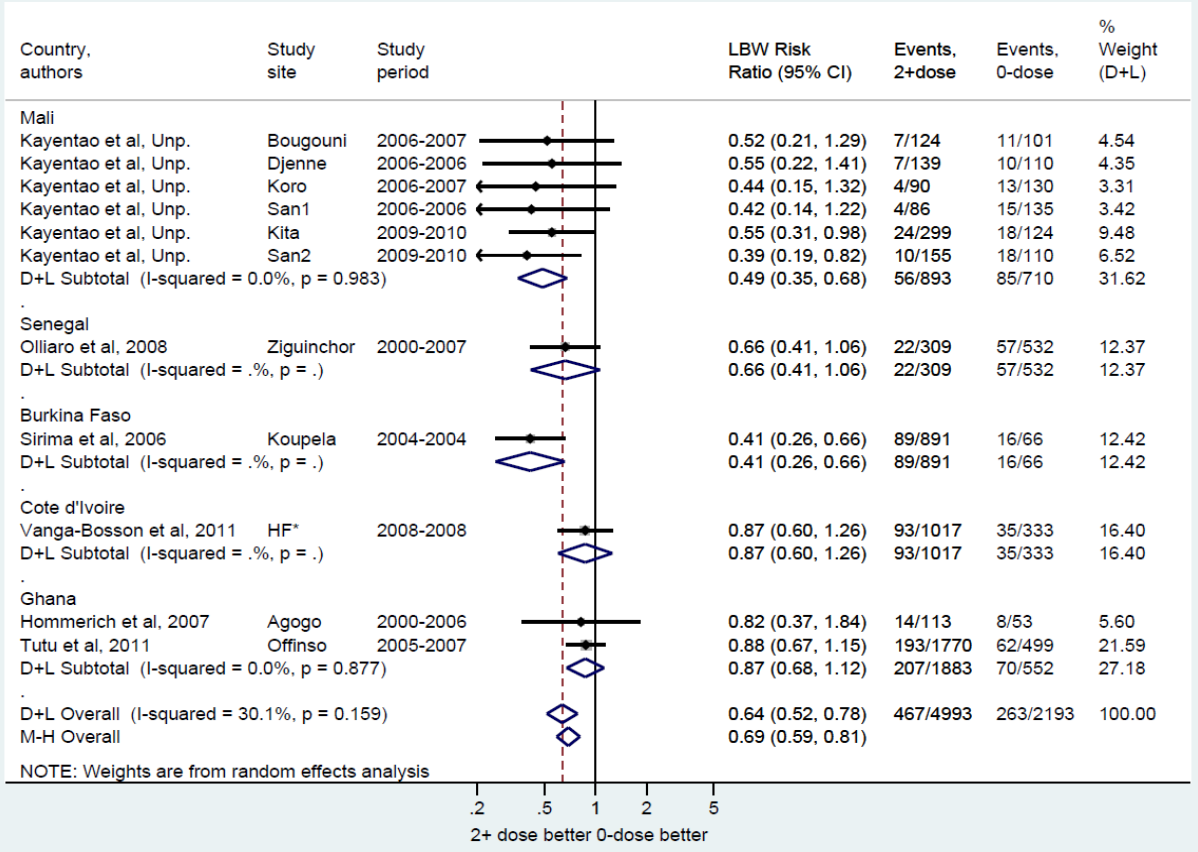
9. Annexes

9.1 Meta-analysis of the risk of low birth weight with the receipt of IPTp-SP in 11 observational studies in 5 countries of West Africa.

The results gathered in chapter 6 were combined with those from similar studies conducted in 4 countries of West Africa. Using a meta-analysis approach, we looked at the prevalence ratio of LBW in women with at least two doses of SP versus those not receiving SP. This showed a pool relative risk reduction of 36% (95% CI, 22-48) in women taking 2 or more doses of SP (Figure 9.1). When plotting the estimates of the relative risk reduction on LBW against the population prevalence of DHPS A437G using meta-regression, a statistically significant linear trend was apparent (Figure 9. 2 and Figure 9. 3) and a greater impact of IPTp-SP was evident in areas with <50% DHPS A437G (RR, 0.50; 95% CI, 50-64) (I^2 =0%, 8 surveys, 3 countries) compared to areas with >50% DHPS A437G (RR, 0.87; 95% CI, 0.71-1.07) (I^2=0%, 3 surveys, 3 countries). Thus, in the presence of very low prevalence of DHPS K540E in West Africa, a high level of the DHPS 437 mutation is a good predictor of waning IPTp-SP effectiveness. This is potentially important observation that illustrates the potential value of regular molecular monitoring of SP resistance in West African countries along with clinical surveys that assess the association between IPTp-SP use and birth parameters.

Annex

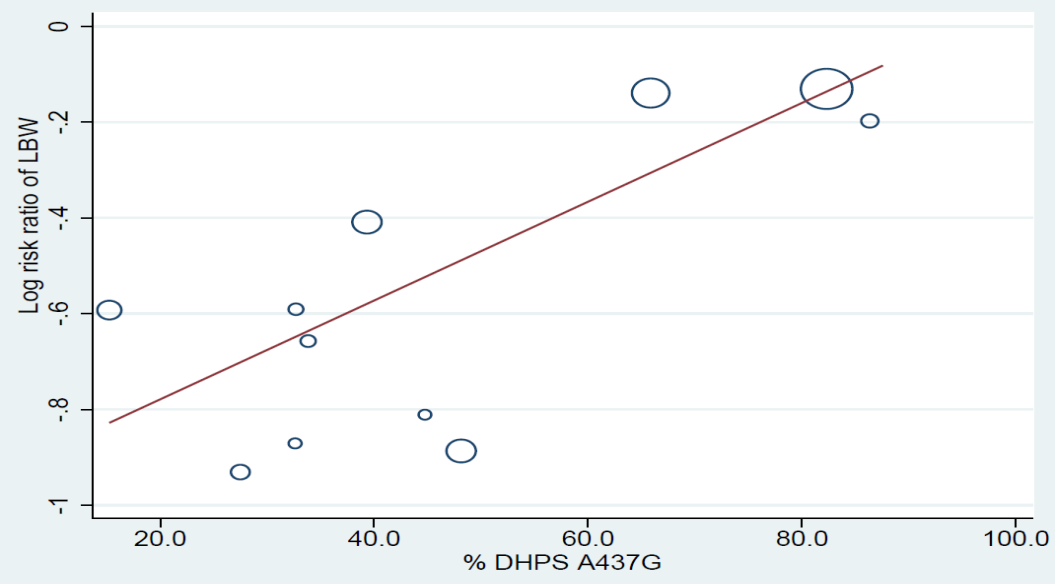
Figure 9. 1: Meta-analysis of the risk of low birth weight associated with the receipt of IPTp-SP in 11 observational studies in 5 countries of west Africa



Notes: LBW, low birth weight; CI, confidence interval; D+L, Dersimonian-Laid methods for random effects models; M-H, Mantel-Haenszel methods for fixed effects models; Unp., unpublished studies. Data marker sizes indicate the weight applied to each study using random-effects meta-analysis. Diamonds represent summary effect of studies. P-values following the I^2 statistics represent the Chi-square test for heterogeneity. Subgroup difference was significant (Chi2 =12.21; P=0.02; I2=67.2%)
HF* Health facilities

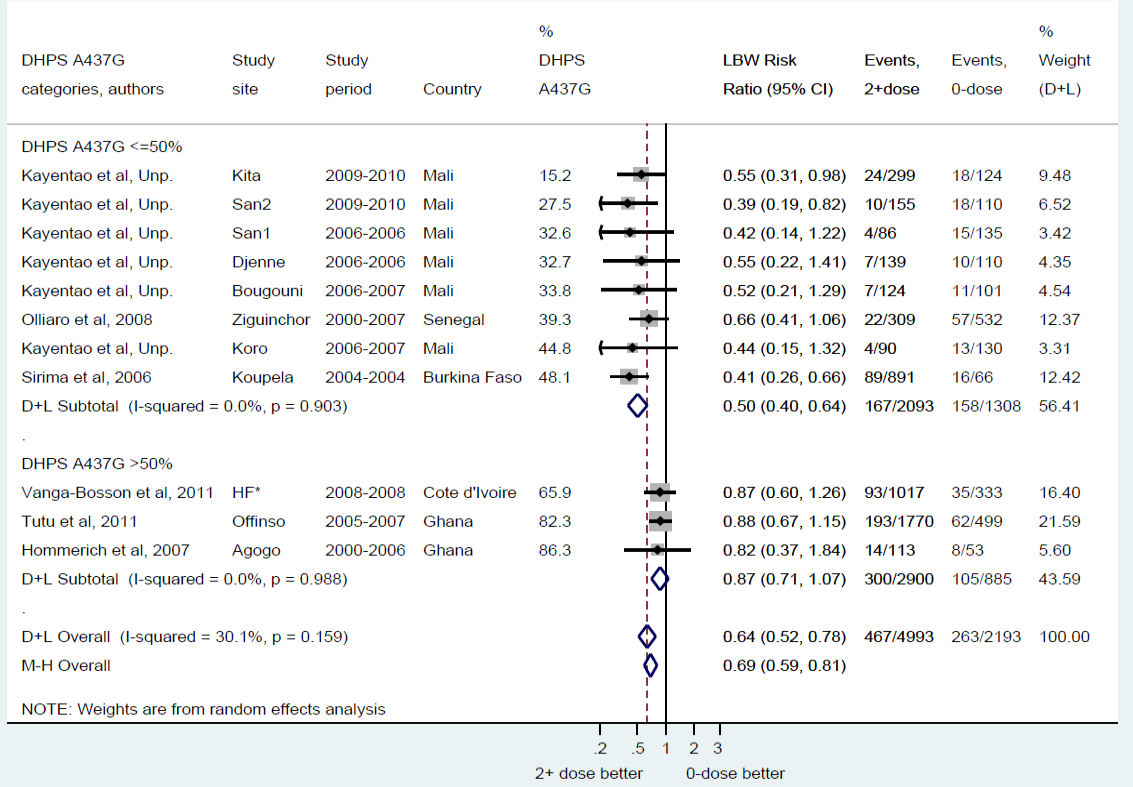
Annex

Figure 9. 2: Meta-regression of the relationship between the prevalence of DHPS A437G and log risk ratio of LBW in 11 observational studies in 5 countries of west Africa



Notes: LBW, low birth weight; DHPS, dihydropteroate synthase; Study specific estimates are depicted as circles proportional to their precision. The solid line indicates fitted values by random-effects meta-regression. P-value for linear trend: P=0.016

Figure 9. 3:Meta-analysis of the risk of low birth weight associated with the receipt of IPTp-SP by DHPS A437G strata in 11 observational studies in 5 countries of west Africa



Notes: DHPS, dihydropteroate synthase; LBW, low birth weight; CI, confidence interval; D+L, Dersimonian-Laid methods for random effects models; M-H, Mantel-Haenszel methods for fixed effects models; Unp., unpublished studies. Data marker sizes indicate the weight applied to each study using random-effects meta-analysis. Diamonds represent summary effect of studies. P-values following the I^2 statistics represent the Chi-square test for heterogeneity.
HF* Health facilities

9.2 Published papers of chapter 3, chapter 4, and chapter 5

MALARIA JOURNAL

RESEARCH Open Access

Parasite clearance following treatment with sulphadoxine-pyrimethamine for intermittent preventive treatment in Burkina-Faso and Mali: 42-day in vivo follow-up study

Sheick O Coulibaly[1], Kassoum Kayentao[2,3*], Steve Taylor[4,5], Etienne A Guirou[2], Carole Khairallah[3], Nouhoun Guindo[2], Moussa Djimde[2], Richard Bationo[1], Alamissa Soulama[1], Edgar Dabira[1], Binta Barry[2], Moussa Niangaly[2], Hammadoun Diakite[2], Sidiki Konate[2], Mohamed Keita[2], Boubacar Traore[2], Steve R Meshnick[5], Pascal Magnussen[6], Ogobara K Doumbo[2] and Feiko O ter Kuile[3]

Abstract

Background: Intermittent Preventive Treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) is widely used for the control of malaria in pregnancy in Africa. The emergence of resistance to SP is a concern requiring monitoring the effectiveness of SP for IPTp.

Methods: This was an *in-vivo* efficacy study to determine the parasitological treatment response and the duration of post-treatment prophylaxis among asymptomatic pregnant women receiving SP as part of IPTp in Mali and Burkina-Faso. The primary outcome was the PCR-unadjusted % of patients with parasites recurrence by day 42 defined as a positive diagnostic test by malaria smear at any visit between days 4 and 42. Treatment failure was based on the standard World Health Organization criteria. The therapeutic response was estimated using the Kaplan-Meier curve.

Results: A total of 580 women were enrolled in Mali (N=268) and Burkina-Faso (N=312) and followed weekly for 42 days. Among these, 94.3% completed the follow-up. The PCR-unadjusted cumulative risk of recurrence by day 42 was 4.9% overall, and 3.2% and 6.5% in Mali and Burkina Faso respectively (Hazard Ratio [HR] =2.14, 95%, CI [0.93-4.90]; P=0.070), and higher among the primi– and secundigravida (6.4%) than multigravida (2.2%, HR=3.01 [1.04-8.69]; P=0.042). The PCR-adjusted failure risk was 1.1% overall (Mali 0.8%, Burkina-Faso 1.4%). The frequencies (95% CI) of the *dhfr* double *and* triple mutant and *dhps* 437 and 540 alleles mutant genotype at enrolment were 24.2% (23.7-25.0), 4.7% (4.4-5.0), and 21.4% (20.8-22.0) and 0.37% (0.29-0.44) in Mali, and 7.1% (6.5-7.7), 44.9% (43.8-46.0) and 75.3% (74.5-76.2) and 0% in Burkina-Faso, respectively. There were no *dhfr* 164L or *dhps* 581G mutations.

Conclusion: SP remains effective at clearing existing infections when provided as IPTp to asymptomatic pregnant women in Mali and Burkina. Continued monitoring of IPTp-SP effectiveness, including of the impact on birth parameters in this region is essential.

Keywords: Malaria, Pregnancy, Intermittent, Sulphadoxine-pyrimethamine, Resistance, Mali, Burkina-Faso

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Background

In sub-Saharan Africa, malaria places 31 million pregnancies at risk of maternal anaemia and intrauterine growth retardation resulting in low birth weight (LBW) annually [1-3]. In this region, the World Health Organization (WHO) recommends Intermittent Preventive Treatment in pregnancy (IPTp) with at least two doses of sulphadoxine-pyrimethamine (SP) for the control of malaria in pregnancy [4]. The two-dose IPTp-SP regimen has been shown to be very effective and is associated with an average reduction in the risk of LBW of 29% [2]. More recent meta-analysis has shown that this can be enhanced further by providing three or more doses of SP at monthly intervals during pregnancy [5].

However, the emergence of SP resistance is potentially reducing the effectiveness of SP. In the early 2000s, SP was abandoned as first line treatment for symptomatic malaria in the general population in sub-Saharan Africa in favour of more effective artemisinin-based combination therapy (ACT). Because IPTp with SP continued to provide significant protection in areas with moderate to high parasite resistance [2], SP continues to be recommended by WHO for IPTp, and is currently the only anti-malarial used for this indication [6]. The degree of SP resistance correlates with the frequency of single nucleotide polymorphisms (SNPs) that encode amino acid substitutions in the dihydrofolate reductase (*dhfr*) and dihydropteroate synthetase (*dhps*) genes of *Plasmodium falciparum*. High grade resistance is a particular concern in eastern and southern Africa [7], where high frequencies of parasites bearing haplotypes with three mutations in *dhfr* (encoding the N51I, C59R, and S108N) and two in *dhps* (encoding the A437G and K540E substitutions) exist, especially if the additional *dhfr*164L or *dhps*581G mutations occur [8,9]. The latter has recently been associated with poor birth outcomes in IPTp-SP recipients [10], although this association has not yet been confirmed in other studies in eastern and southern Africa [11-13].

In contrast, the parasite populations in western Africa seem to be mostly sensitive to SP [7,14-16], and IPTp-SP has proven to be highly effective and efficacious in clinical trials and observational studies [5,15]. However, spread of SP resistance from eastern and southern Africa, or the *de novo* development of high-level SP resistance may occur and monitoring of the effectiveness of SP when employed as IPTp is essential.

Despite this need, there are no internationally standardized methods to evaluate the *in vivo* effectiveness of IPTp-SP. Furthermore, the relationship between the level of SP resistance as measured by molecular markers and impact of IPTp-SP on birth parameters, or the treatment response in asymptomatic women receiving SP for IPTp is not known. Hitherto, monitoring SP resistance was predominantly based on *in vivo* treatment responses among symptomatic children with acute malaria. However, extrapolation from children to asymptomatic pregnant women is not appropriate as protective immunity against *P. falciparum* malaria is acquired progressively with cumulative exposure and age. As a result pregnant women in endemic areas remain typically asymptomatic when infected and have lower parasites densities than sick children and as a results have better treatment responses to anti-malarials, including SP [17,18]. A single arm 42 days *in vivo* efficacy study of SP was conducted to determine the parasitological treatment response to SP and the duration of post-treatment prophylaxis among asymptomatic parasitaemic women receiving SP for IPTp in, Mali and Burkina Faso. The prevalence of molecular markers for SP resistance was also assessed to explore the relationship between the level of resistance and the treatment responses.

Methods

Study sites and study period

In Mali, the study was conducted from July 2009 to March 2010 in 2 district health centres located in the towns of Kita in the Kayes region in western Mali and in San in the Segou region situated approximately 500 kilometres east of Kita (Figure 1). Malaria transmission in the two sites is typical for most of the Sahel region with highly seasonal transmission restricted to a single period of three to five months during and shortly after the rainy season, with peak transmission in October. The degree of SP resistance is low in these areas and the quintuple *dhfr/dhps* haplotype has not been found yet [15], although the *dhps*581G mutation has been described in isolation of other mutations in other settings [19,20]. No previous in-vivo studies among pregnant women were conducted in Mali.

In Burkina Faso, the study was conducted from January 2010 to December 2011 in five recruitment centres in Ziniaré town, Oubritenga Province, located 400 km South-East of the town of San (one of the study sites in Mali). Malaria transmission is seasonal peaking in September-October. In 2003, the polymerase chain reaction (PCR)-adjusted parasitological failure rate by day-28 was 13% among symptomatic primi- and secundigravida with acute falciparum malaria in Ouagadougou, located 50 kilometers from the study site [16].

Participants and procedures

In both countries, pregnant women of all parities with a gestational age between 16–30 weeks attending for antenatal care for their first dose of IPT-SP were included. Women were screened for malaria infection using HRP2 and pLDH-based combo Rapid Diagnostic Tests (RDTs, CareStart™Malaria HRP-2/pLDH[Pf/pan] Combo Test) [21,22]. Women with a positive RDT were then screened

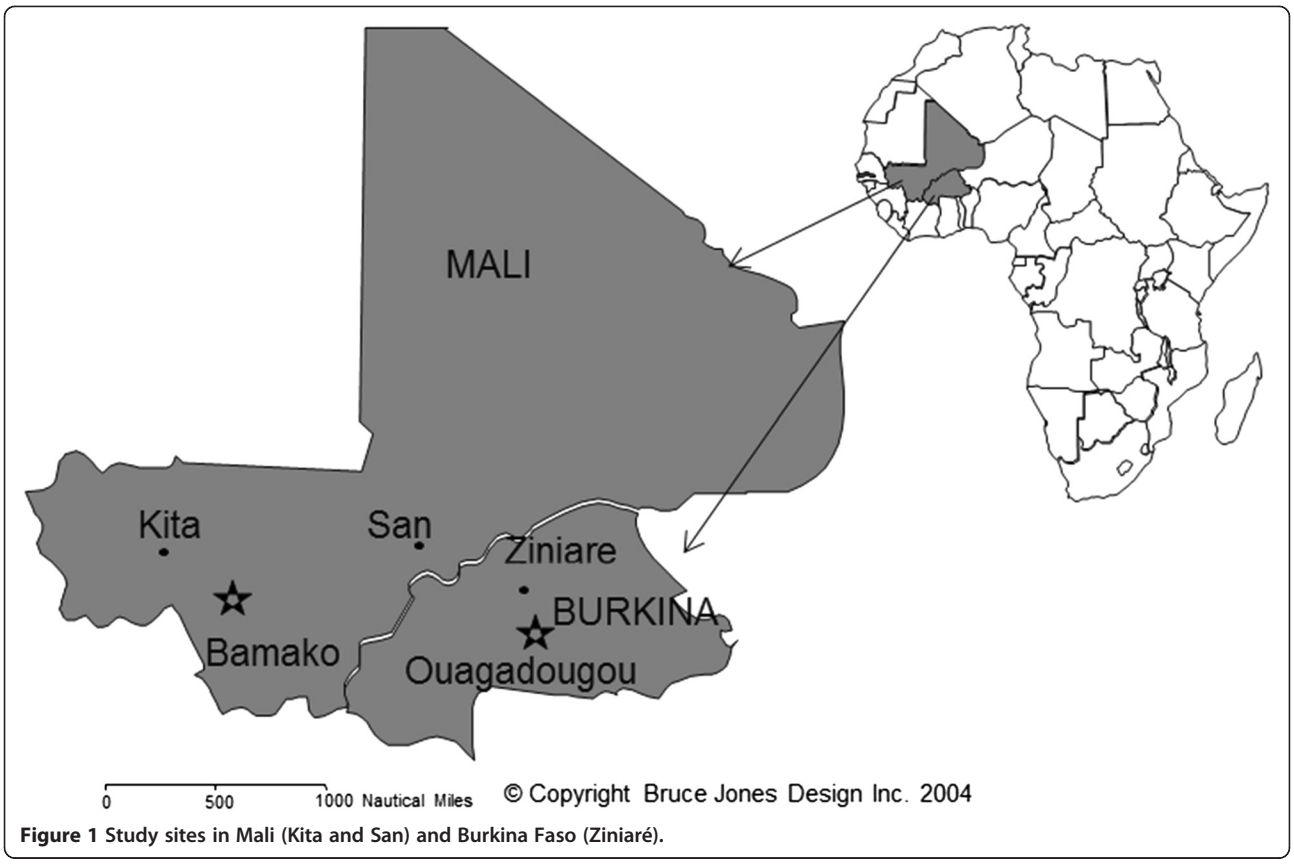

Figure 1 Study sites in Mali (Kita and San) and Burkina Faso (Ziniaré).

for malaria parasitaemia by microscopy and eligible for enrolment if they had a positive blood smear, were asymptomatic, were willing to participate in the six-week follow-up and provided written individual informed consent. Women were excluded if they had a history of hypersensitivity to SP or its components, a history of prior use of IPTp-SP during this pregnancy, or a history of receipt of other anti-malarials or antibiotics with anti-malarial activity in the previous month.

On enrolment, clinical, obstetric and demographic data were obtained and information on bed net type and use recorded. A finger-prick blood sample was taken for malaria smears, haemoglobin assessment, and dried blood spots (DBSs) for parasite DNA.

Three tablets of SP containing a total dose of 1,500 mg sulphadoxine and 75 mg of pyrimethamine were administered as a single dose on day 0 by the study staff. If vomiting occurred within 30 minutes after administration, the full dose was re-administered. Women were scheduled to be seen again weekly from day 7 onwards for 42 days for a brief clinical exam, assessment of the axillary temperature and collection of blood by finger prick for malaria smears, RDT, and DBSs for PCR. Participants were asked to return to the study clinic any time they felt ill in between the scheduled visits. Women with positive smear or severe malaria at any time on or after day 4 were treated according to national guidelines.

In Mali, the study drug used was manufactured by Kinapharma limited Ltd, Ghana and in Burkina Faso this was also from Kinapharma limited Ltd, Ghana and Medreich limited, India. A sample of 50 tablets from each batch was assessed for quality using high-performance liquid chromatography (HPLC) conducted in Atlanta, GA, USA by the US Centers for Disease Control and Prevention (CDC) to determine the amount of the active ingredient and the dissolution profile. Both brands passed the dissolution and content analyses criteria set by the United States Pharmacopeia (USP).

Laboratory methods

Haemoglobin concentrations were measured using HemoCue® (301 System) on days 0, 14, 28 and 42, and on the day of parasite recurrence. Giemsa-stained blood smears were assessed in duplicate and if a discrepancy was found (positive *vs* negative) the smear was read by a third expert microscopist. Asexual parasites were counted against 300 leukocytes and densities expressed per mm^3 of blood assuming a leucocyte count of 7,500/mm^3. Smears were declared negative if no parasites were detected in 100 high-power fields.

PCR assays were performed in the laboratories of the Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, USA using genomic DNA (gDNA) extracted from dried blood spots (DBSs) stored on Whatman 3 MM filter papers to differentiate between recrudescence and new infection in follow-up specimens with parasite recurrence. A standard method was employed to genotype parasites using polymorphisms of the merozoite surface protein-1 (*msp-1*), merozoite surface protein-2 (*msp-2*), and glutamate rich protein (*glurp*) genes [23]. The prevalence of genomic markers of parasite SP resistance, genomic DNA from all parasitaemic women was pooled by study site (2 in Mali and 1 in Burkina Faso). Fragments of the *dhfr* and *dhps* genes containing the SNPs of interest were PCR-amplified from the pooled gDNA from each site to produce a mixture of gene fragments [24], and these PCR products were sequenced on a Roche GS Junior next-generation sequencing system.

Study endpoints classification

The primary outcome was the PCR-unadjusted % of patients with parasites recurrence by day 42, defined as a positive diagnostic test (by microscopy) for malaria at any visit between days 4 and 42. To define treatment failure, the standard WHO criteria [25] were used.

Statistical analysis

Data were analysed using STATA v12 and SPSS version 20. The treatment responses are summarized by weeks of follow-up. The therapeutic response was estimated using the Kaplan-Meier product limit formula [26]. In the PCR-unadjusted analysis, recurrences were treated as treatment failures and all other events (e.g. withdrawal or protocol deviations) resulted in censoring at the time of that event, or at the time of their last follow-up visit in case of loss to follow-up. A similar strategy was used for the PCR-adjusted analysis except that

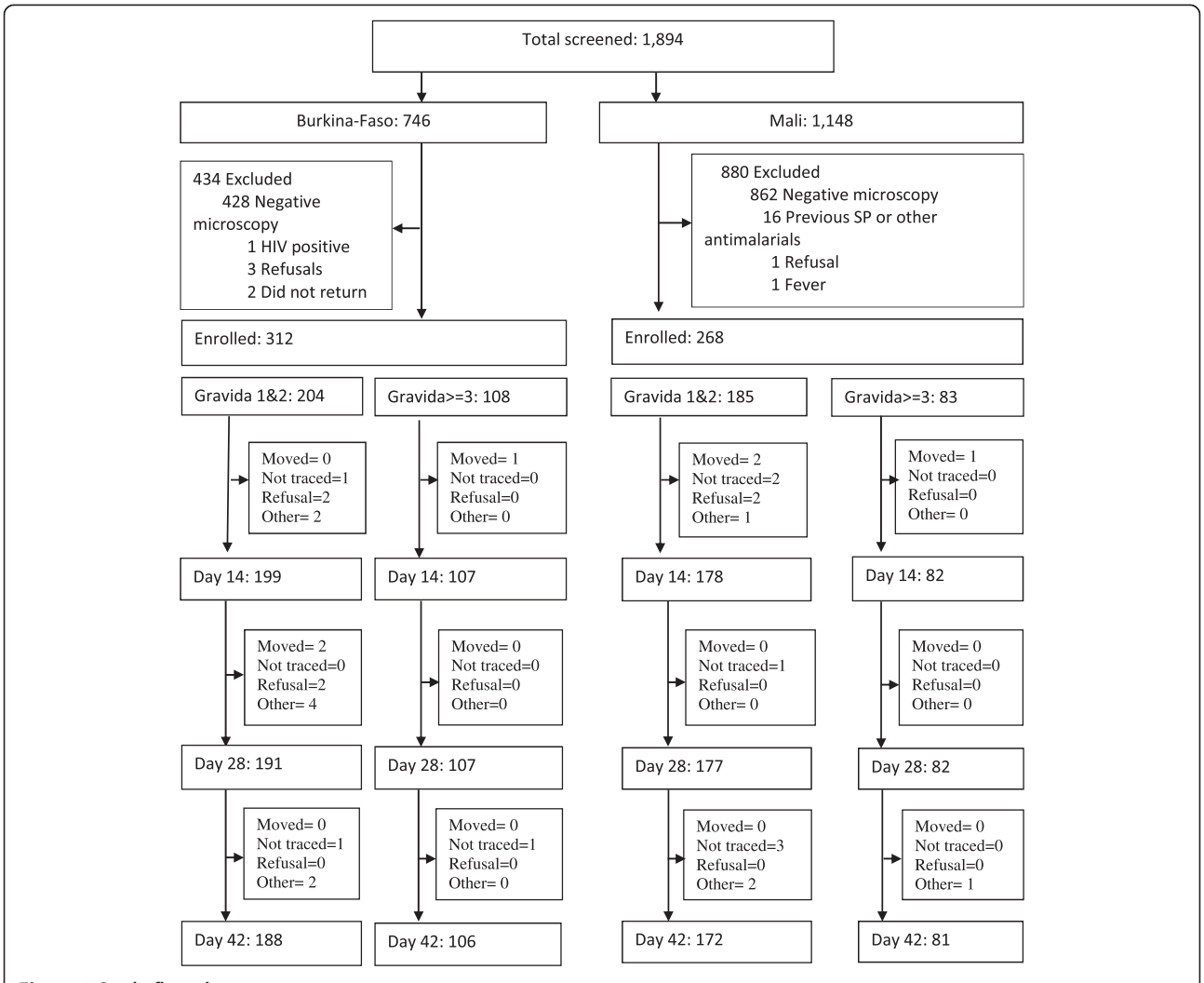

Figure 2 Study flow chart.

patients with new *P. falciparum* infections (reinfections) were censored at the time of parasite reappearance [26].

Ethical considerations

The protocol was approved by the Faculty of Medicine, Pharmacy and Dentistry, University of Bamako, Mali, Institutional Ethical Review Committee, the National Ethical Review Committee and Ministry of Health, Burkina-Faso, the University of North Carolina, USA, and the Liverpool School of Tropical Medicine, UK.

Results

Treatment responses

Overall 580 of 584 women who fulfilled all enrollment criteria were enrolled (99.3%, Figure 2 and Table 1), and 572 of the 580 contributed to the survival analysis. Eight of the 33 women lost to follow-up were not seen after day 0; 3 from Mali and 5 from Burkina-Faso. The remaining 25 were censored on the day they were last seen.

PCR-unadjusted efficacy: Based on microscopy, overall 27 of the 572 women had a recurrence of parasitaemia by the end of follow-up (Mali 8; Burkina Faso 19). The cumulative recurrence risks by day 42 estimated by survival analysis were 4.9% overall, and 3.2% and 6.5% in Mali and Burkina Faso respectively (Hazard Ratio [HR] Burkina vs Mali=2.14, 95% CI 0.93-4.90; P=0.070, Table 2 and Figure 3). The recurrence risk was higher among primi- and secundigravidae (6.4%) than multi-gravidae (2.2%), HR=3.01 (1.04-8.69; P=0.042) (Figure 4).

PCR-adjusted efficacy: From 26 of the 27 recurrences, DNA could be extracted and 24 were genotyped successfully. This suggested that only 6 of the 24 were recrudescences. The PCR-adjusted cumulative failure rate obtained by survival analysis was 1.1% overall, and 0.8% in Mali and 1.4% in Burkina-Faso (Figure 3). Overall, median (range) time to PCR-adjusted failure and to reinfection was 21 (7–35) and 35 (7–43) days, respectively.

Haematological response: There was a significant increase in the mean haemoglobin concentrations compared to enrolment at all-time points measured in both countries and both among primi-, secundi- and multigravida (Figure 5 and Table 3).

Table 1 Baseline characteristics of women enrolled in SP *in vivo* efficacy study, Burkina-Faso and Mali

	Burkina-Faso N=312	Mali N =268	All N=580
Age, years,			
Mean (SD)	23.6 (5.4)	21.1 (5.1)	22.5 (5.4)
Residing in rural area, n (%)	81 (30.2)	146 (45.2)	222 (38.3)
Knows the date of LMP, n (%)	44 (16.4)	61 (19.6)	105 (18.1)
Pregnancy number			
Median (range)	2 (1–8)	2 (1–9)	2 (1–9)
First or second pregnancy, n (%)	204 (65.4)	185 (69.0)	389 (67.1)
Use of a bed net last night[a]			
Any net, n (%)	187 (60.1)	207 (77.2)	394 (68.1)
ITN, n (%)	171 (55.0)	180 (67.2)	351 (60.6)
Use medicine in first trimester			
Any medicine, n (%)	6 (1.9)	25 (9.3)	31(5.3)
Antimalarial, n (%)	2 (0.6)	15 (5.6)	17 (2.9)
Fundal height, cm			
Mean (SD)	21.5 (2.9)	21.8 (3.2)	21.7 (3.1)
Gestational age, weeks			
Mean (SD)	25.3 (3.1)	25.4 (3.2)	25.3 (3.1)
Maternal height, cm			
Mean (SD)	162.7 (6.2)	162.2 (6.3)	162.4 (6.2)
Maternal weight, kgs			
Mean (SD)	57.8 (7.5)	56.4 (8.5)	57.2 (8.0)
Haemoglobin, g/dL[b]			
Mean (SD)	10.1 (1.4)	9.6 (1.6)	9.9 (1.5)
Anaemia (Hb <11 /dL), n (%)	225 (72.4)	198 (80.5)	423 (75.9)
Moderate-Severe anaemia (Hb <8g/dL),	22 (7.1)	40 (16.3)	62 (11.1)
n (%)			
Peripheral parasitaemia	623	716	664
GMPD/µl (95% CI)	(537–723)	(598–859)	(592–746)

Notes: Data are n0. (%), unless otherwise indicated.
N, sample size; n, number of events; SD, Standard deviation; LMP, Last Menstrual Period; ITN, Insecticide Treated Net; cm, centimeters; kgs, kilograms; g/dL, Gram per deci-Litre; Hb, Haemoglobin; GMPD/µl, Geometric Mean Parasite Density per microlitre.
[a]Bed net use was not evaluated in 1 subject from Burkina-Faso.
[b]Haemoglobin was not measured for 1 subject in Burkina-Faso and 22 subjects in Mali.

Prevalence of molecular markers for SP resistance at booking

No *dhfr* 164L or *dhps*581G mutations were found in any of the three sites; the *dhps* 540E mutation was found in one site of the two sites Mali, but at a very low prevalence (Figure 6).

Discussion

SP when given as IPTp to asymptomatic parasitaemic pregnant women was associated with a high cure rate and marked increases in haemoglobin concentrations by day 42 in the 3 study sites in Mali and Burkina-Faso. Overall, only 4.9% of women had a recurrence of parasites by day 42, and genotyping suggested that the vast majority of these were reinfections. Overall only 1.1% of treatments resulted in true treatment failures (recrudescence) and all of these were asymptomatic. The study shows that SP remains very effective at clearing existing infections when used as IPTp for malaria prevention in Mali and Burkina-Faso. This study also showed the potential value of using in-vivo follow-up to assess the parasitological cure rates

Table 2 Parasitological efficacy of SP among women enrolled in Burkina-Faso and Mali

Characteristics	Burkina-Faso		Mali		All	
	N=312		N =268		N =580	
PCR Days	Non- adjusted	Adjusted	Non- adjusted	Adjusted	Non- adjusted	Adjusted
Day 7:						
Number at risk	307	307	265	265	572	572
Failures, n (%)						
ETF	0	0	0	0	0	0
LCF	0	0	0	0	0	0
LPF	2 (0.5)	1 (0.3)	1 (0.4)	1 (0.4)	3 (0.5)	2 (0.3)
ACPR n (%)	305 (99.5)	306 (99.7	264 (99.6)	264 (99.6)	569 (99.5)	570 (99.7)
Day 14:						
Number at risk	306	306	260	260	566	566
Failures n (%)						
ETF	0	0	0	0	0	0
LCF	0	0	0	0	0	0
LPF	3 (1.0)	2 (0.7)	1 (0.4)	1 (0.4)	4 (0.7)	3 (0.5)
ACPR n (%)	303 (99.0)	304 (99.3	259 (99.6)	259 (99.6	562 (99.3)	563 (99.5)
Day 21:						
Number at risk	300	300	259	259	559	559
Failures n (%)						
ETF	0	0	0	0	0	0
LCF	0	0	0	0	0	0
LPF	3 (1.0)	2 (0.7)	1 (0.4)	1 (0.4)	4 (0.7)	3 (0.5)
ACPR n (%)	297 (99.0)	298 (99.3)	258 (99.6)	258 (99.6)	542 (99.5)	556 (99.5)
Day 28:						
Number at risk	297	297	259	259	556	556
Failures n (%)						
ETF	0	0	0	0	0	0
LCF	0	0	0	0	0	0
LPF	7 (2.4)	3 (1.0)	2 (0.8)	2 (0.8)	9 (1.6)	5 (0.9)
ACPR n (%)	290 (97.6)	294 (99.0)	257 (99.2)	257 (99.2)	547 (98.4)	551 (99.1)
Day 35:						
Number at risk	295	294[a]	253	254	548	547[a]
Failures n (%)						
ETF	0	0	0	0	0	0
LCF	0	0	0	0	0	
LPF	14 (4.8)	4 (1.4)	2 (0.8)	2 (0.8)	16 (2.9)	6 (1.1)
ACPR n (%)	281 (95.2)	290 (98.6)	251 (99.2)	252 (99.2)	532 (97.1)	541 (98.9)
Day 42:						
Number at risk	293	292[a]	253	251[a,b]	546	544[c]
Failures n (%)						
ETF	0	0	0	0	0	0
LCF	0	0	0	0	0	0

Table 2 Parasitological efficacy of SP among women enrolled in Burkina-Faso and Mali *(Continued)*

LPF	19 (6.5)	4 (1.4)	8 (3.2)	2 (0.8)	27 (4.9)	6 (1.1)
ACPR n (%)	274 (93.5)	288 (98.6)	245 (96.8)	249 (99.2)	519 (95.1)	538 (98.9)
Median (range) time in days	35 (7–43)[d]	21 (7–35)[e]	42 (7–42)[d]	18 (7–29)[e]	35 (7–43)[d]	21 (7–35)[e]

Notes: PCR, Polymerase Chain Reaction; ETF, Early Treatment Failure; LCF, Late Treatment Failure; LPF, Late parasitological Failure; ACPR, Adequate Clinical and Parasitological Response.
[a]One PCR inconclusive.
[b]PCR analysis not conducted for 1 woman with recurrent parasitaemia in Mali.
[c]PCR inconclusive (1 from Mali, 1 from Burkina) and no PCR analysis available (1 from Mali). These three cases were censored in the survival analysis.
[d]Median (range) time to reinfection.
[e]Median (range) time to PCR-adjusted failure.

among parasitaemia asymptomatic pregnant women who are due for their first dose of SP for IPTp.

The pooled molecular assays for the surveillance of SP resistance showed that almost 50% of the parasite population in Burkina Faso, but only 9% in San and <1% in Kita, carried the *dhfr* triple mutations. The pooled deep sequencing of *P. falciparum* parasitaemia can provide estimates of the mutant allele frequencies, but does not provide estimates of the quadruple and quintuple *dhfr/dhps* haplotypes. Nevertheless, the *dhps* 540E mutation, which is a

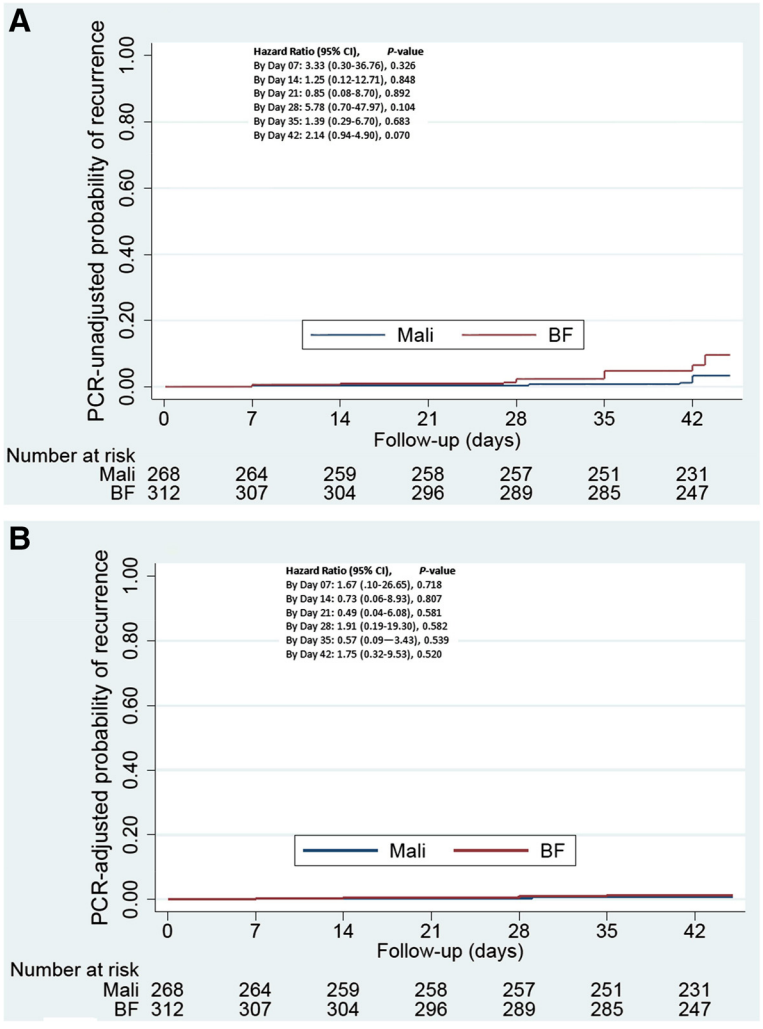

Figure 3 Probability of parasitological failure by microscopy in Burkina-Faso and Mali. Notes: This graph shows the crude and PCR adjusted risk of parasitological failure in Mali and Burkina-Faso. Treatment failure was defined according to the standard WHO criteria and the cumulative risk of recurrence was determined using Kaplan-Meier survival analysis. Blue lines represent Mali and red lines Burkina-Faso. Panel **A** and panel **B** represent survival analysis for crude and PCR adjusted analysis, respectively.

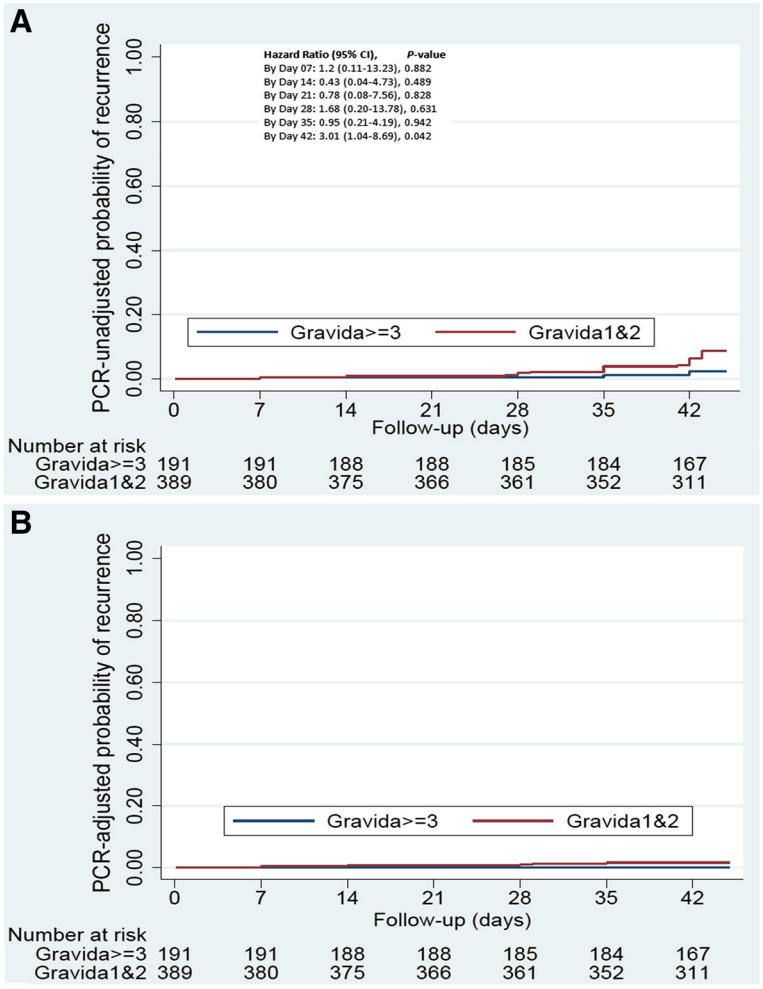

Figure 4 Probability of parasitological failure by microscopy by gravida group. Notes: (Gravidae 1&2, primi-secundigravida; Gravidae>=3, multigravida): PCR unadjusted **(Panel A)** and PCR adjusted **(Panel B)**. This graph shows the crude and PCR adjusted risk of parasitological failure in both primi-secundigravida and multigravida using Kaplan-Meier survival analysis. Blue lines represents multigravida (gravidae>=3) and the red lines represent primi-secundigravida (gravidae 1&2), respectively.

proxy for the quintuple haplotype conferring mid-level resistance to SP, was present in only one of the two sites of Mali and at very low frequency (0.73%, 95% CI 0.58-0.87). The mutation at *dhfr* codon 164L and *dhps* codon 581G conferring very high-level resistance to SP were absent. In addition, there were several novel mutations in *dhps,* which were limited to a very low frequency. Their clinical and biological significance is unknown, but their quantification underscores the ability of the pooled genotyping approach to uncover low-level sub-populations of parasites.

The 1.4% failure rate in Burkina Faso among asymptomatic women in this study is in contrast to the 13% PCR-adjusted failure rate by day-28 observed in the previous in-vivo study among symptomatic pregnant women conducted in 2003 in an area located ≈32 miles south from the current site [16]. The average parasite densities in the previous study were 10 fold higher than in the current study, illustrating the differences in treatment responses when SP is used as IPTp in asymptomatic women with predominantly low-grade parasitaemia *vs.* acutely ill women requiring case-management drugs. This may in part explain the earlier findings from randomized controlled trials that IPTp-SP remained surprisingly effective in areas with moderate to high levels of SP resistance [2,5].

The study provides an important contribution to the understanding of the predictive value of the frequency of population estimates of the different *dhfr* and *dhps* mutations on the efficacy of SP in clearing malaria infection among asymptomatic pregnant women, especially when our results are compared against day 42 failure rates in areas with higher resistance. For example, the *dhfr* triple mutation (Ile51+Arg59+Asn108) was present in almost

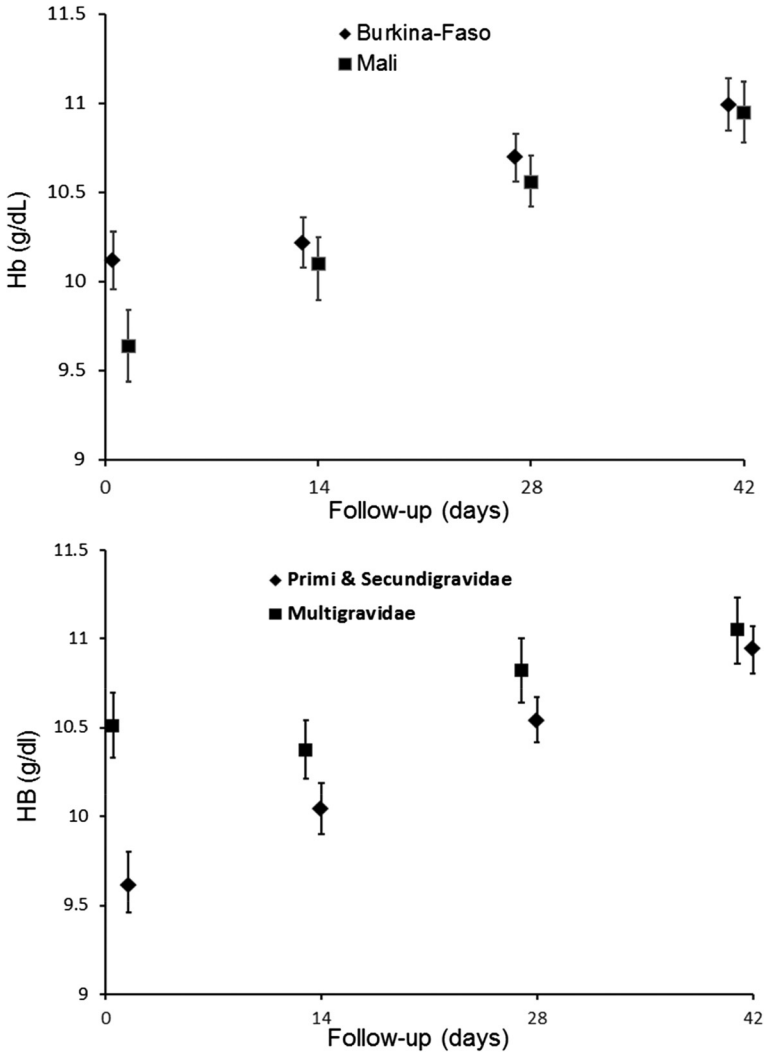

Figure 5 Increase in haemoglobin concentrations by country in all gravida. Notes: (top panel) and by gravidae group (bottom panel). Analysis was done with repeated measures Generalized Estimating Equation (GEE), adjusted for the baseline hemoglobin levels on Day-0. Black squares or diamonds represent the point estimates and vertical lines the corresponding 95% confidence intervals.

50% of the parasite population in Burkina Faso yet only 1.4% of the treatments recrudesced by day 42. The *dhfr* triple mutation is known to confer intense pyrimethamine resistance in vitro [27] and is associated with an approximate 1,000-fold reduction in pyrimethamine susceptibility [28] and with an increased risk of SP treatment failure in children with acute malaria [29-31]. These combined data suggests that parasite densities and immunity contribute importantly to parasite clearance, which in turn influences the association of treatment outcome with *dhfr* and *dhps* alleles.

It is likely that the results of this study are representative for large parts of West and Central Africa that have a similar low geographic prevalence of the *dhfr/dhps* quadruple or quintuple mutations reflecting low and mid-level resistance to SP [7]. A key question is whether this situation can be sustained or whether further development of SP drug resistance is inevitable in this region. Mutations arise under antifolate pressure in a stepwise fashion, with successive mutations conferring higher levels of resistance [32]. Previous studies from Ghana showed a rapid increase in the prevalence of the triple-mutant *dhfr* alleles among *falciparum* isolated from pregnant women in an area where pyrimethamine prophylaxis (as mono-therapy) was used 6 to 8 years previously for the prevention of malaria [33]. Some fitness-reducing mutations, such as the *dhfr* I164L can only be sustained under conditions of sustained drug pressure. The switch from SP as first-line treatment for symptomatic malaria in the general population to an ACT will have hada marked impact on reducing SP drug

Table 3 Haemoglobin concentration and anaemia among women enrolled in Burkina-Faso and Mali

Characteristics	Burkina-Faso	Mali	All
Day 0			
N	311	246	557
Mean haemoglobin (SD), g/dl	10.1 (1.4)	9.6 (1.6)	9.9 (1.5)
Anaemia (<11 g/dl), n (%)	225 (72.4)	198 (80.5)	423 (75.9)
Day 14			
N	301	237	538
Mean haemoglobin (SD), g/dl	10.2 (1.3)	10.1 (1.4)	10.2 (1.3)
Anaemia (<11 g/dl), n (%)	208 (69.1)	177 (74.7)	385 (71.6)
Mean difference, 95% CI[a]	0.13 (0.004, 0.26)	0.44 (0.28, 0.59)	0.26 (0.16, 0.36)
Risk ratio, 95% CI[b]	0.96 (0.86, 1.06)	0.93 (0.84, 1.02)	0.94 (0.88, 1.01)
Day 28			
N	290	244	534
Mean haemoglobin (SD), g/dl	10.7 (1.2)	10.6 (1.2)	10.6 (1.2)
Anaemia (<11 g/dl), n (%)	171 (59.0)	153 (62.7)	325 (60.7)
Mean difference, 95% CI[a]	0.60 (0.46,0.74)	0.87 (0.69, 1.06)	0.72 (0.61, 0.83)
Risk ratio, 95% CI[b]	0.82 (0.72, 0.92)	0.78 (0.70, 0.87)	0.80 (0.74, 0.87
Day 42			
N	265	249	514
Mean haemoglobin (SD), g/dl	11.0 (1.2)	10.9 (1.3)	10.9 (1.3)
Anaemia (<11g/dl), n (%)	127 (47.9)	120 (48.2)	247 (48.1)
Mean difference, 95% CI[a]	0.87 (0.65, 1.09)	1.30 (1.11, 1.49)	1.06 (0.93, 1.18)
Risk ratio, 95% CI[b]	0.66 (0.57, 0.77)	0.60 (0.52, 0.69)	0.63 (0.57, 0.70)

Notes:
N, sample size; n, number of events; SD, standard deviation; g/dl, gram per decilitre; CI, confidence interval.
[a]Mean difference and 95% confidence interval for each time that haemoglobin was measured using day 0 as reference category.
[b]Risk ratio and 95% confidence interval for each time that haemoglobin was measured using day 0 as reference category, adjusted for gravida and site (all), and for gravida (in each country).

pressure in the population [34]. However, the effect of continued use of cotrimoxazole in the treatment of diarrheal and respiratory infectious diseases in children should also be considered, although this drug did not appear to select for SP-resistance parasites [19]. Modelling of the impact of the introduction of IPTi in infants suggest that use of SP in small target populations such as infants or pregnant women may not sustain sufficient drug pressure to impact on the spread of drug resistance. This was also suggested in field studies in Mali [35]. However, many West African countries including Mali and Burkina are seeking to implement Seasonal Malaria Chemoprevention (SMC) [36] in children which would provide presumptive treatment over the course of the transmission season to a much larger fraction of the population. Although, the combination of amodiaquine (AQ) and SP is one of the main candidate anti-malarials for SMC, it is unclear if the introduction of this strategy will indeed increase SP drug pressure. The effect of SMC on drug pressure may be minimal if implemented on a large enough scale to impact on malaria transmission and the total parasite biomass in the SMC population, especially if an ACT is used as case-management for clinical episodes caused by any SP resistant parasites that may have escaped the drug action of SMC. It will be clearly important to monitor the prevalence of molecular markers of parasite resistance to SP, especially in areas where SP is used for both IPTp and SMC.

This investigation found a high prevalence of anaemia and showed that SP treatment was associated with a marked increase in mean haemoglobin levels by day 42. The fact that the impact was most pronounced in the primi,- and secundigravidae, the group most susceptible to adverse effect of malaria, may indicate that even these asymptomatic infections are an important cause of maternal anaemia in this subgroup. These findings are consistent with previous findings that showed IPTp has a marked beneficial impact on moderate-to-severe anaemia in Mali [15,37].

The study was limited by the lack of genotyping of parasites from individual women for molecular markers of SP resistance, and the genomic DNA from pooled sequencing

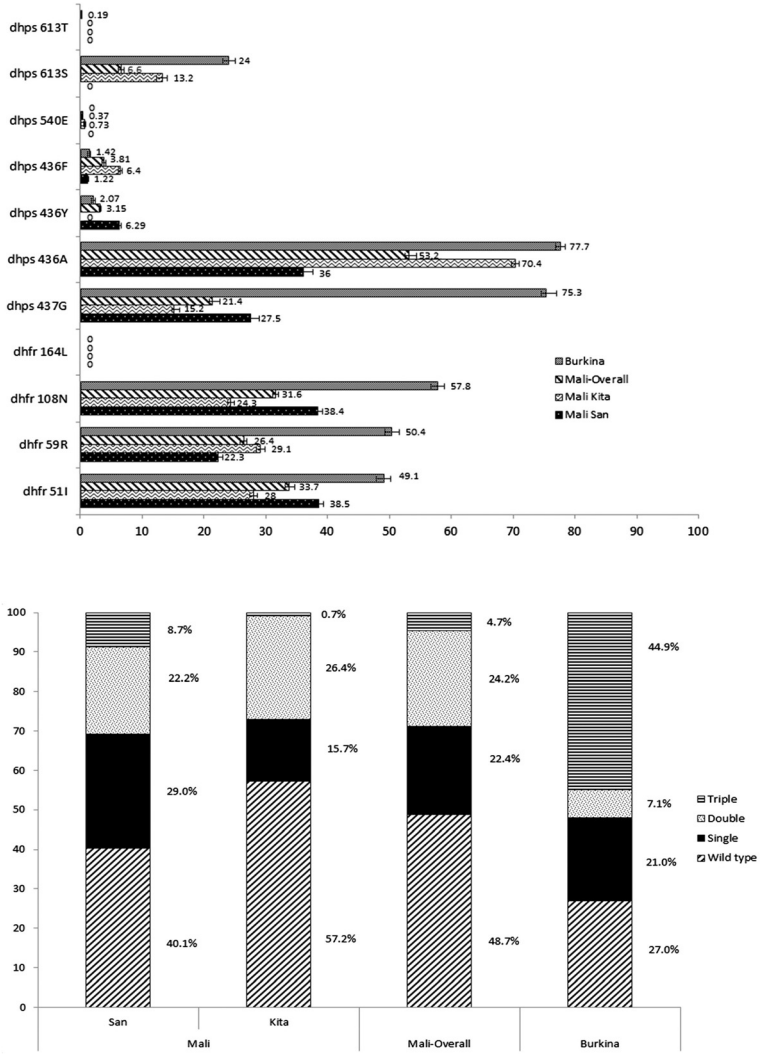

Figure 6 Prevalence of SP resistance molecular makers in Burkina-Faso and Mali among parasitaemic women at their antenatal booking visit (pre-SP). Notes: *dhfr /dhps* alleles (Top panel) and *dhfr* haplotypes (Bottom panel). Mutant allele frequencies are represented in the top panel graph by horizontal bars. Lines depict the 95% confidence intervals. The presence of "0" represents the absence of point mutations for a designed codon. The bottom panel represents the frequency of *dhfr* haplotypes (N51I, C59R, and S108N) per country.

by study site was not able to explore the correlation between treatment efficacy or the haematological response in individual women and SP resistance molecular markers.

Conclusion

This is among the first studies to examine the 42-day *in vivo* response of IPTp-SP in asymptomatic women in areas with low level of SP resistance in West Africa. Despite growing concerns about the impact of SP resistance in east and southern Africa, this study shows that SP remains effective at clearing existing infections and improving haemoglobin concentration when provided as IPTp to asymptomatic pregnant women in Mali and Burkina-Faso. SP has many attributes that makes it an excellent candidate for IPTp, and it is thus likely that it could remain the drug of choice for IPTp in this region for the foreseeable future. However continued monitoring of SP resistance over the next years in this region coupled with monitoring of IPTp-SP effectiveness on birth parameters is essential.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KK and FtK designed the study and drafted the manuscript. SOC and KK were the principal investigators in Burkina-Faso and Mali, respectively and contributed equally to the study. The field work in Burkina Faso was conducted by RB, AS, and ED, and in Mali by EAG, NG, MD, BB, MN, HD, SK, MK. BT was the field supervisor in Mali. ST and SM were responsible for the molecular component of the study, data interpretation, and manuscript

writing. CK helped with data analysis and results interpretation. PM, OKD, supported the field work and were involved with data interpretation and manuscript writing. All the authors reviewed the final version of the manuscript.

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